Identification of Mechanisms and Pathways Involved in MLL2-Mediated Tumorigenesis

by

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Thesis submitted in partial fulfillment of
the requirements for the degree of
Master of Science in the Department of
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ABSTRACT

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Abstract

Myeloid/lymphoid or mixed-lineage leukemia (MLL)-family genes encode histone lysine methyltransferases that play important roles in epigenetic regulation of gene transcription, and these genes are frequently mutated in human cancers. While MLL1 and MLL4 have been the most extensively studied, MLL2 and its homolog MLL3 are not well-understood. Specifically, little is known regarding the extent of global MLL2 involvement in the regulation of gene expression and the mechanism underlying its alterations in mediating tumorigenesis. To study the role of MLL2 in tumorigenesis, we somatically knocked out MLL2 in a colorectal carcinoma cell line, HCT116. We observed that the MLL2 loss of function results in significant reduction of cell growth and multinuclear morphology. We further profiled MLL2 regulated genes and pathways by analyzing gene expression in MLL2 wild-type versus MLL2-null isogenic cell lines. Our results reveal the connection of MLL2 to multiple cellular signaling pathways and suggest potential mechanisms underlying tumorigenesis mediated by MLL2 alterations.

Dedication

I dedicate my thesis work to my beloved family. I especially feel gratitude to my loving parents, Chih-Wen Chang (張志文) and Lien-Chih Chang (張蓮枝), who always support me with words of encouragement. My husband Tzung-Lian Tzeng (曾宗廉) and my sister Chun-Ping Chang (張君萍) have never left my side. I also dedicate this thesis to my friends who have supported me throughout the process. I will always appreciate all they have done, especially Christopher Pirozzi for helping me the many hours of proofreading. I dedicate this work and give special thanks to my church family for being there pray for me throughout the entire master program. All of you have been my best cheerleaders.

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1. Introduction

Modulation of chromatin accessibility through histone methylation is a critical step in regulating eukaryotic gene transcription. Histone H3 lysine 4 (H3K4) methylation by histone methyltransferases is an epigenetic mark for active gene transcription [1]. In human cells, four Mixed-Lineage Leukemia (MLL) family proteins have H3K4 methyltransferase activity, each with an enzymatic Su (var) 3–9 Enhancer-of-zeste Trithorax methyltransferase (SET) domain. These MLL-family genes include MLL1, MLL2 (also known as ALR/MLL4), MLL3, and MLL4 (also known as MLL2 or Wbp7, conflicting with the official symbol for MLL2). Each of these proteins is associated with multiple subunits that function as a complex with histone-modifying activities [2]. Among the MLL genes, MLL1 and its homologue, MLL4, have been the most extensively studied. MLL1 is frequently involved in genetic alterations in acute myeloid and acute lymphoblastic leukemia [3]. Overexpression of MLL4 has been found in breast and colorectal cell lines and primary tissues [4].

MLL2 was originally cloned as a human homolog of *Drosophila trithorax* [5]. MLL2 and its homolog, MLL3, function distinctly from MLL1 and MLL4. Both MLL2 and MLL3 associate with nuclear receptor coactivator 6 (NCOA6, also known as Activating Signaling Cointegrator-2, ASC-2) to form a complex that contains other essential subunits, including ASH2L, RbBP5, WDR5, DPY30, PTIP, PA1, and UTX, a histone H3K27 demethylase [6-8]. The MLL2 or MLL3 (hereafter named MLL2/MLL3) complex has been found to play an essential role as a coactivator for transcriptional activation by nuclear hormone receptors [9]. Previous studies have shown that

MLL2/MLL3 complexes regulate Homeobox (Hox) gene transcription, and that they play critical roles in PPARγ-dependent adipogenesis [10-12].

With the latest high-throughput next generation sequencing, new cancer driver genes previously unlinked to human cancers have been discovered. Among these genes, the MLL2/MLL3 pathway is the most frequently mutated driver in an ever-growing list of cancers. Initially, our medulloblastoma (MB) exome sequencing revealed frequent cancer driver inactivating mutations of histone lysine methyltransferase gene MLL2, and its paralogue gene MLL3. These were altered in 16% of cases (passenger gene probability <0.001), a prevalence which is comparable to classic MB cancer genes such as CTNNB1 (12%). Most somatic MLL2 gene mutations identified in MBs lead to frame-shift. [13]. Subsequent comprehensive studies involving larger numbers of well-classified MB samples have significantly extended these genetic findings and further confirmed that dysregulation of the MLL2/MLL3 pathway, including mutations in a MLL2 and MLL3-associated demethylase, UTX, plays an important role in driving various groups of human MBs [14-16]. These results clearly established the aberrant MLL2/MLL3 pathway as a bona fide cancer-driving pathway.

In addition to its role in MB development, recent studies found that the MLL2/MLL3 pathway is frequently mutated in other cancers (summarized in Table 1), including 89% of follicular lymphoma and 20%-30% of diffusive large B-cell lymphoma [17, 18]. MLL3 is also mutated in colorectal cancer [19, 20]. Other cancers that have recently been found to be driven by the aberrant MLL2/MLL3 pathway, mainly through MLL2 or MLL3 mutations, include renal [21], prostate [22] , bladder [23], gastric [24], liver [25], pancreatic [26], and lung [27, 28]. UTX was also found to be mutated in a variety of human cancers with various frequencies [29]. Furthermore, germ line MLL2 inactivation has recently been found to be the major cause of Kabuki syndrome [30], a rare pediatric congenital disorder characterized by intellectual disabilities. Collectively, these findings place the MLL2/MLL3 pathway among the most frequently mutated pathogenic pathways that drive human cancers.

In contrast to the well-documented genetic evidence about MLL2/MLL3 mutations, much less is known regarding the mechanism underlying the tumor suppressor roles of the MLL2/MLL3 pathway. A recent study showed that shRNA-mediated knockdown of MLL2 resulted in reduced cancer cell growth and altered adhesion in HeLa cells, although the underlying mechanism and its relevance to tumorigenesis were unknown [7]. To further elucidate the role of MLL2 in tumorigenesis, we utilized recombinant adeno-associated virus (rAAV) to somatically knockout MLL2 in the colorectal cancer cell line, HCT116. Knockout of MLL2 in human cells affects the expression of a variety of genes. We note a set of genes that are retinoic acid-responsive and may be directly regulated by MLL2. Finally, integrative pathway analysis uncovered various signaling pathways that are regulated by the MLL2 complex. Our results reveal the global and pathway-specific roles of MLL2 and suggest that it participates in regulating a wide range of pathways with relevance to its role in oncogenesis [31].

Table 1. Genes in the MLL2 pathway are frequently mutated in various

Cancer	Gene (mutation frequency)	References
Medulloblastoma	MLL2, MLL3, UTX (~12-16%)	[13-16]
Non-Hodgkin's lymphoma	MLL2(89% follicular lymphoma; 20-30% Diffusive large B cell lymphoma)	[17, 18]
Colorectal cancer	MLL3 (~14% of microsatellite deficient CRCs)	[19, 20]
Renal cancer	MLL, MLL2, UTX (~7%)	[21]
Prostate cancer	MLL2, MLL3, UTX (~24%) (castration-resistant)	[22]
Bladder cancer	MLL, MLL3, UTX (~40% of transitional cell carcinoma)	[23]
Gastric cancer	MLL, MLL3 (~20%)	[24]
Liver cancer	MLL, MLL3 (~33%)	[25]
Pancreatic cancer	MLL3 (8%) (ductal adenocarcinoma)	**[26]**
Lung cancer	MLL2 and MLL3 (`10-20%) (squamous cell)	[27,28]
Various cancer types	UTX (various frequencies)	[29]

2. Materials and Methods

2.1 Cell Culture

Human colorectal carcinoma cell line HCT116 (American Type Culture Collection) was cultured in McCoy's 5A medium (Gibco) with 10% (vol/vol) FBS (HyClone). Cells were grown up to 70% confluency in 100 × 20 mm cell culture dishes (Corning) for genomic DNA, RNA, or nuclear protein extraction. HCT116-MLL2$^{-/-}$ cells were maintained in complete medium containing 0.5 mg/mL Geneticin. HEK 293FT cells (Invitrogen) were cultured in DMEM with high glucose (Gibco) containing 10% (vol/vol) FBS and used for rAAV targeting virus production as previously described [32]. Exponentially growing cells were used for microarray gene expression profiling. For retinoic acid treatment, ~10^6 cells in six-well plates were treated with 1 μM all-trans retinoic acid (Sigma) for 48 hours before cells were harvested for RNA preparation.

2.2 Somatic Targeting

The vector and protocol for epitope tag targeting and gene knockout have been described previously [33-35] . To generate MLL2-knockout cell lines, we utilized a rAAV vector to insert a stop codon right before the SET domain (amino acid 5391Leu), which is the enzymatic functional domain of MLL2. The rAAV vector was composed of two homology arms which were ∼1–1.2 kb in length and surrounded the insertion site, and a neomycin (neo) selection marker surrounded by two loxP sites. The homology arms were amplified from HCT116 genomic DNA using Hotstart High-Fidelity Platinum Taq polymerase (Invitrogen).

Using Lipofectamine 2000 (Invitrogen), the vector was co-transfected with pAAV-RC and pHelper plasmids (Invitrogen) into HEK 293FT cells to generate rAAV targeting virus using a modified procedure based on the previously described protocol [33, 35]. Virus was harvested 2 days after transfection. HCT116 cells were infected with targeting virus for 5 hours. Cells were allowed to recover in complete culture medium for 2 days, and were serially diluted into 96-well plates in culture media containing 0.5 mg/mL Geneticin. Geneticin-resistant cells were grown for 14–21 days, harvested for Lyse-N-Go genomic DNA extraction (Thermo Scientific), and screened by PCR using two sets of primers (see Table 2 for primer information). The first set of primers has the forward primer, P1, located in the genome, upstream of the left homologous arm, and the reverse primer, NR, is located in the neo marker insertion region. The second set of primers has the forward primer, NF, located in the neo marker insertion region, and reverse primer, P2, located downstream of the right homologous arm in the genome. The screening PCR products that identified positive clones were confirmed by sequencing (Genewiz).

Positive clones were then infected with Cre recombinase-expressing adenovirus (Vector Biolabs) to remove the drug-resistance selection marker. Cells were serially diluted and cultured in complete media for 10–14 days, and clones were picked for PCR screening using primers P3 and P4 (see Table 2 for primer information).

After the generation of $MLL2^{+/-}$ cell lines, two independent $MLL2^{+/-}$ lines were used for targeting the second MLL2 allele in exactly the same manner to generate $MLL2^{-/-}$ cell lines. The neomycin selection marker in the second MLL2 allele was not removed. MLL2 knockout ($MLL2^{-/-}$) was confirmed by genotyping, sequencing (Genewiz) the mutant transcript (RT-PCR), and anti-MLL2 immunoblot.

2.3 Nuclear Extraction

Chemicals were ordered from Sigma unless otherwise stated. Briefly, ~1 x 10^7cells were harvested, washed with buffer A (10 mM Hepes·KOH (EMD), pH 7.9, 1.5 mM magnesium chloride, 10 mM potassium chloride, 0.5 mM DTT, 5 mM sodium fluoride, 2 μM sodium orthovanadate (MP Biomedical), protease inhibitor cocktail (Roche)), and lysed in buffer A with 0.1% Nonidet P-40. The nuclei were collected by centrifugation (4 °C, at 3500 g for 5 min) and lysed in high-salt buffer C (20 mM Hepes·KOH, pH 7.9, 1.5 mM magnesium chloride, 420 mM sodium chloride, 0.2 mM EDTA (Gibco), 25% (vol/vol) glycerol, 0.5 mM DTT, 5 mM sodium fluoride, 2 μM sodium orthovanadate, protease inhibitor mixture) at 4 °C on rotating mixer. Nuclear lysate was directly used for immunoblot.

2.4 Western Blot

Nuclear extract was resolved on 4–12% SDS/PAGE using NuPAGE system (Invitrogen). The blot was transferred on a piece of nitrocellulose paper using a TE22 tank transfer unit (Hoefer) filled with transfer buffer (1x NuPAGE transfer buffer, 0.1% SDS, 5% methanol, 8.75mM 2-Mercaptoethanol) under 400mA or 100V at 4 °C for 4 hours. Nitrocellulose papers with the blots were then blocking with 1% casein in Tris-buffered saline (TBS, 50mM Tris-HCl, pH 7.5, 150mM NaCl) (Bio-rad) at 4 °C for overnight. The blots were stained with 1:1000 diluted anti-MLL2 antibody (gift from Dr. Kai Ge [6]) in blocking buffer at room temperature for at least 2 hours, and wash with TBS with 0.05% Tween-20 (TBST) for 10 mins, 3 times. HRPconjugated goat anti-rabbit or anti-mouse IgG (1mg/mL) were diluted 1:5000 in the blocking buffer, and incubate with the blots for 0.5 hours at room temperature, and wash with TBST for 10 mins, 3 times. The blots were developed with SuperSignal West Femto Chemiluminescent Substrate (Pierce). The membrane was exposed to film for 0.5-10 mins.

2.5 MTT Assay

MTT assay was used to determine the cell viability and cell growth. About 10^4 cells were plated in 96-well plate on day 0, and MTT assay was performed daily for 4 days to evaluate the overall proliferation of the cells. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; Sigma) was dissolved in PBS at 5 mg/ml and filtered with 0.2 um filter for sterilization. On each day, 10ul of stock MTT solution were diluted in 100ul fresh culture medium, and added into the wells to be tested. The plates were incubated at 37°C for 4 hours. After incubation, 200 ul of acid-isopropanol (1 portion of 4N HCl : 100 portion of isopropanol) was added to all wells and mixed by pipetting thoroughly to dissolve the dark blue crystals. The plates were read on a POLARstar Optima microplate reader, using a test wavelength of 570 nm to determine the relative cell viability. Plates were normally read within 1 hours of adding the isopropanol.

2.6 Microarray Assay

Total RNA was prepared from MLL2$^{-/-}$ cells (two pairs of cell lines, clone 1, 2 and cell line clone 3, 4, which were derived from two independent MLL2$^{+/-}$ progenitor lines, were included for two separate microarray experiments) and HCT116 parental cells. The quality of the RNA was assessed with a 2100 Bioanalyzer G2939A (Agilent Technologies) and Nanodrop 8000 spectrophotometer (Thermo Scientific). Hybridization targets were prepared with a MessageAmp Premier RNA Amplification Kit (Applied Biosystems/Ambion) from total RNA, hybridized to GeneChip U133 Plus 2.0 arrays in Affymetrix GeneChip Hybridization Oven 645, washed in Affymetrix GeneChip Fluidics Station 450, and scanned with Affymetrix GeneChip Scanner 7G according to standard Affymetrix GeneChip hybridization, wash, and stain protocols. Each cell line was done in triplicate. Statistical analysis for false discovery rate was performed by significance analysis of microarrays [36]. Statistical analysis for the enrichment of down-regulated genes in the ChIP-identified genes was performed by χ^2 test.

2.7 Confirmation of Microarray Gene Expression

The expression change of selected genes was confirmed by RT-qPCR in the original microarray- analyzed pair of MLL2$^{-/-}$ cell lines (clones 1 and 2) and in additional MLL2$^{-/-}$ clones (clones 3 and 4) derived from two independent MLL2$^{+/-}$ progenitor lines. Total RNA was prepared from parental cell lines and MLL2$^{-/-}$ cells using a total RNA kit (Omega Bio-Tek) according to the manufacturer's instructions. One microgram of total RNA was used for reverse transcription using a SuperScript II First-Strand Synthesis Kit (Life Technologies) according to the kit manual. After transcription, the cDNA was diluted 1:20 and used as template for qPCR.

SYBR green-based real-time qPCR was performed with an Applied Biosystems 7900 platform (Life Technologies). Quantitative PCR was done in 20-μL reactions containing 1× PCR buffer (67mMTris·HCT,pH8.8, 6.7mMMgCl2, 16.6 mM NH4SO4, 10 mM 2-mercaptoethanol), 2 mM dNTPs, 0.5 μM forward and reverse primers, 6% (vol/vol) DMSO, 1:2,000 SYBR green, and 1 unit Platinum Taq (Invitrogen). The PCR program was 95 °C, 10 min for hotstart, amplification for 38 cycles (95 °C, 15 s to 60 °C, 15 s to 72 °C, 15 s), and a dissociation curve analysis (95 °C, 15 s to 60 °C, 15 s to 95 °C, 15 s, ramp rate 2%).Gene expression levels were normalized to the housekeeper gene GAPDH (see Primers for list of primers used for qPCR).

2.8 Microarray Data Analysis

The web-based Ingenuity Pathway Analysis (IPA) (www.ingenuity.com) tool was used to analyze the genes down-regulated by >50% in the microarray. Top affected canonical pathways were identified for the down-regulated genes (Table 3).

2.9 Chromatin Immunoprecipitation (ChIP) and ChIP-Quantitative PCR

The ChIP procedure is described in detail in our previous publication. Briefly, cells were cross-linked and cell lysate was then prepared and subjected to sonication to shear the chromatin. Magnetic beads (New England BioLabs) conjugated with anti-Flag antibody (F3165; Sigma) were used for IP. ChIP-derived DNA was recovered for Illumina GAII-compatible library preparation. Libraries were used for sequencing or qPCR analysis. High-throughput sequencing was performed on the Illumina GAII system. Tags were mapped to the human genome (release hg18) by using the Illumina alignment software Eland.

2.10 ChIP-Quantitative PCR

Primers were designed according to the enriched locus coordinates, and SYBR green-based real-time qPCR was performed with an Applied Biosystems 7900 platform (Life Technologies). A LINE-1 amplicon was used for normalization (refer to Table 2 of primers used for ChIP-qPCR).

ChIP DNA preparation was done as described above. ChIP DNA (2.5 μL) was used as qPCR template. Quantitative PCR was done in 20-μL reactions containing 1×PCRbuffer (67mMTris·HCT,pH8.8, 6.7mMMgCl2, 16.6 mM NH4SO4, 10 mM 2-mercaptoethanol), 2 mM dNTPs, 0.5 μM forward and reverse primers, 6% (vol/vol) DMSO, 1:2,000 SYBR green, and 1 unit Platinum Taq (Invitrogen). The PCR program was 95 °C, 10 min for hotstart, amplification for 38 cycles (95 °C, 15 s to 60 °C, 15 s to 72 °C, 15 s), and a dissociation curve analysis (95 °C, 15 s to 60 °C, 15 s to 95 °C, 15 s, ramp rate 2%).

2.11 Retinoic Acid Treatment

HCT116 parental cells or MLL2$^{-/-}$ cells were treated with retinoic acid (1 μM) for 48 h. Afterward, RNA preparation, reverse transcription to generate cDNA, and real-time qPCR were performed as described above to measure the expression of ASB2 as a readout for retinoic acid treatment.

Table 2. Primers Information

Primers

Name	Direction	Sequence (5'-3')
MLL2-knockout genomic DNA screening		
P1	Forward	TCTGTGTACCAGGGACCTTAGTC
NR	Reverse	GGTCTTCTCGTCCATACTAGTGC
NF	Forward	AGGGGATCGGCAATAAAAAG
P2	Reverse	GCTACCTCTCTTCCCCCTCA
P3	Forward	CTTGGTCTGAGGGAGAGCTG
P4	Reverse	TGATGGTGCCAATGTACTCG
MLL2-knockout RT-PCR primers	Forward	CACTACAAACGGCCCCATAC
	Reverse	TTCTAGGTCCTTGGCTGCAT

ChIP-qPCR primers

Target gene	Direction	Sequence
S100A2	Forward	CCCCTCTACCTCAGCCCTAA
	Reverse	GGTCTCTGTGCCAGTGCTTT
S100A2	Forward	GCACTTCACTGCACACCAAA
	Reverse	GAGGGAGCTCCAAAGAGAGG
S100A4	Forward	CATTCTTTTCCCTCCCAGAA
	Reverse	CTGCAGCTTCTCTTCCAACC
ASB2	Forward	GAATGTGTCACCGTCACCTG
	Reverse	CGAGAGGCTAGGAGCACTGT
BCL9L	Forward	AGTCTCCGAAGACACCTGGA
	Reverse	GGTACTAGCAAGCCCCCTTC
LINE-1	Forward	AAGGCCGCTCAACTACATGG
	Reverse	TGCTTTTGAATGCGTCCCAGAG

3. Results

3.1 Generation of Isogenic MLL2-Knockout Colorectal Cancer Cells

To investigate the function of MLL2 and identify downstream events that were regulated by MLL2, microarray analysis of gene expression in the isogenic MLL2 deficient cells was performed. Two technical barriers have complicated our task: (1) the massive size of MLL2 (5537 amino acid; MW=~600kD) makes gene engineering challenging; and (2) the lack of high quality antibodies. In addition, si-RNA and sh-RNA did not show significant knockdown in our cell lines. To overcome these technical challenges, we have applied recombinant adeno-associated virus (rAAV)-based somatic knockin technology [33, 35] to insert a stop codon into a coding exon of the MLL2 gene in HCT116 cell line. We chose the HCT116 carcinoma cell line for our study, as it is a near-diploid cell line with well-defined key cancerous pathways. Furthermore, this cell line has homozygous MLL3-inactivating mutations, thus preventing compensatory effects from obscuring functional analysis. Using rAAV based somatic knock-in technology, we generated MLL2-null HCT116 cell lines, and effectively engineered inactivated MLL2 mutations similar to those that are frequently found in cancers (Fig. 1). As expected, cell lines with insertions in both MLL2 alleles ($MLL2^{-/-}$) had complete loss of the wild-type MLL2 genes and transcript, and had no detectable MLL2 protein due to the nonsense mutation and/or altered splicing (Fig. 2).

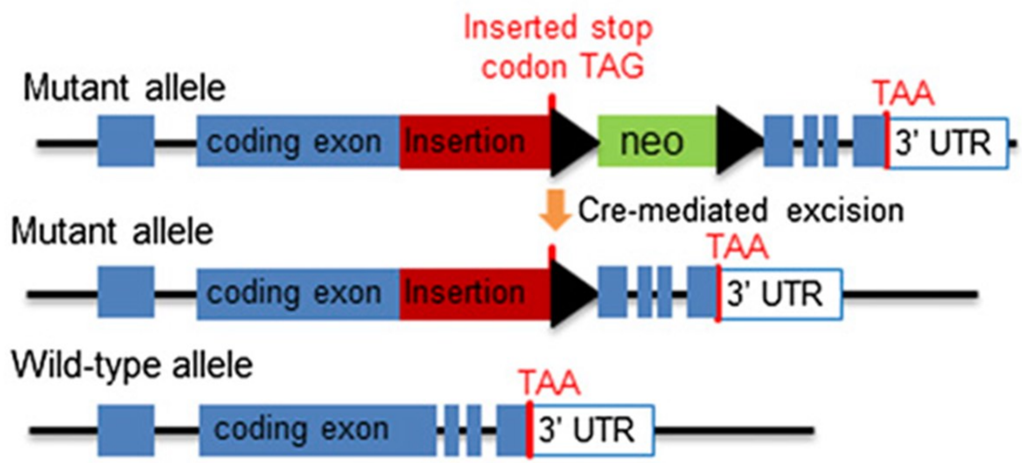


Fig 1. rAAV vector and somatic MLL2 knockout by insertion. A DNA fragment containing a stop codon and a neomycin (neo) selection marker was inserted at the position right before the SET domain-coding sequence. The insertion disrupts MLL2 with or without the presence of the neo selection marker.

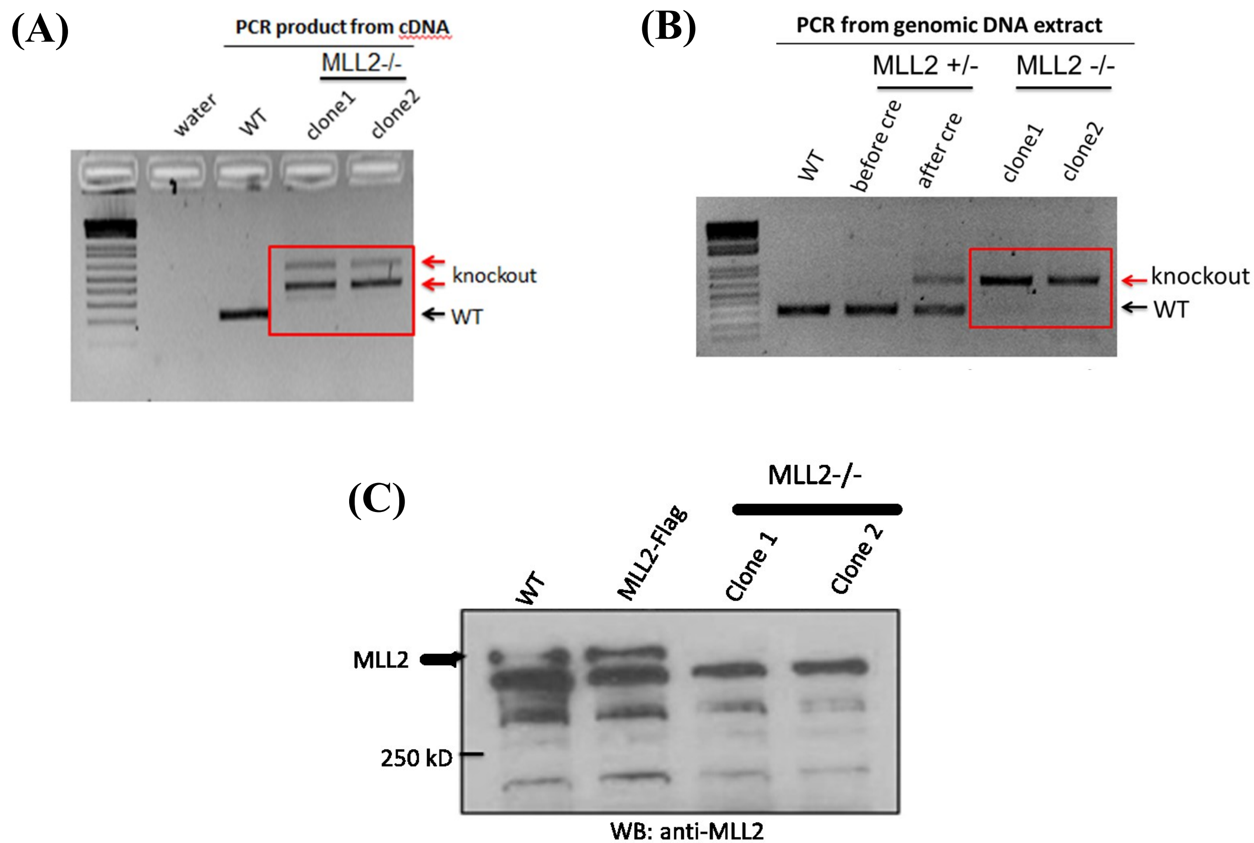


Fig 2. Confirmation of MLL2-/- clones. (A) Detection of insertion in genome by PCR. (B) Detection of insertion in transcript by RT-PCR. (C) Anti-MLL2 westernblot confirms the absends of the expected full-length MLL2 protein in MLL2-/- cells.

3.2 Cell Growth Reduced in MLL2-knockout Cells

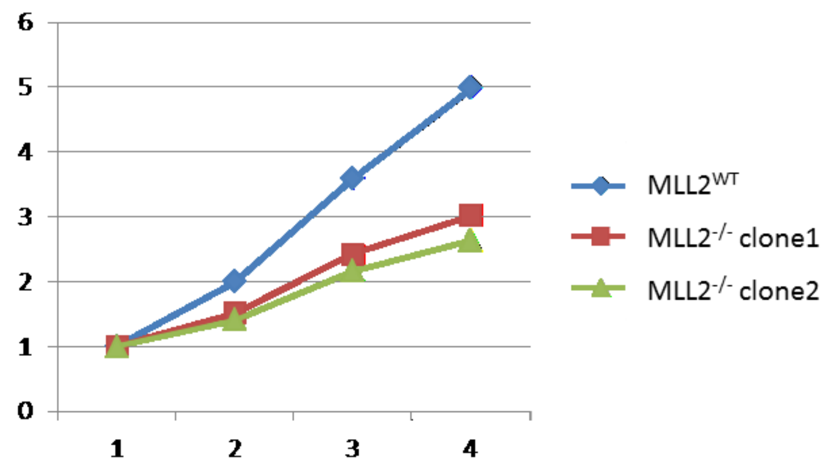
To determine the biological function of MLL2, we observed the morphology and growth in MLL2 deficient cells. MTT assay was used to determine the cell growth in our MLL2-null cells compared with the parental HCT116. Our results showed an overall slower growth of the MLL2$^{-/-}$ HCT116 cell lines (Fig 3A), consistent with the previous result of MLL2 knockdown in HeLa cells [7]. Further, the MLL2$^{-/-}$ cells frequently displayed a flattened morphology and a multinucleated phenotype (Fig 3B). These results indicated that MLL2 deficiency plays roles in cell cycle defect and/or cell senescence.

3.3 Gene Expression Analysis of Parental and MLL2-knockout Cells.

To identify downstream events that were regulated by MLL2, microarray analysis was used to analyze gene expression in the parental HCT116 cells and four MLL2$^{-/-}$ clones. Overall, while a list of genes were identified to be downregulated by MLL2 loss of function (Supplementary Dataset 1), most of the down-regulation of gene expression was moderate, in agreement with the observation that deletion of a single MLL-family gene has only minimal effects on global H3K4 methylation [37, 38]. Interestingly, there were also genes that displayed higher expression levels in MLL2$^{-/-}$ cells than in the parental cells (Fig 4), consistent with an indirect effect. Gene expression changes were further verified by qPCR of transcripts from the four MLL2$^{-/-}$ clones.

To identify MLL2 direct transcriptional target genes, we further identified the MLL2 binding profile and gene expression profile. Our analysis revealed that a subset of genes associated with MLL2-bound loci [31] displays reduced expression in MLL2$^{-/-}$ cell lines (Supplementary Dataset 1), including S100 alpha (S100A) families (Fig 5A ad 5B). The decreased expression was accompanied by reduced H3K4 trimethylation (Fig 5C), consistent with the effect of MLL2 loss of function; while minor or no change in H3K27 trimethylation (Fig 5D).

(A)



(B)

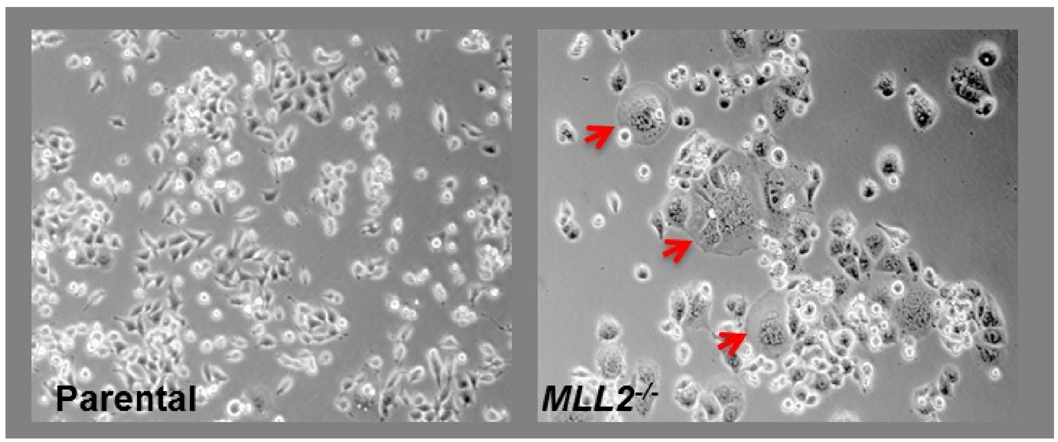


Fig 3. MLL2 deficiency have defective cell growth in HCT116. (A) Knockout MLL2 resulted in reduced cancer cell growth and (B) multinucleated phenotype (red arrows) in HCT116 cells.

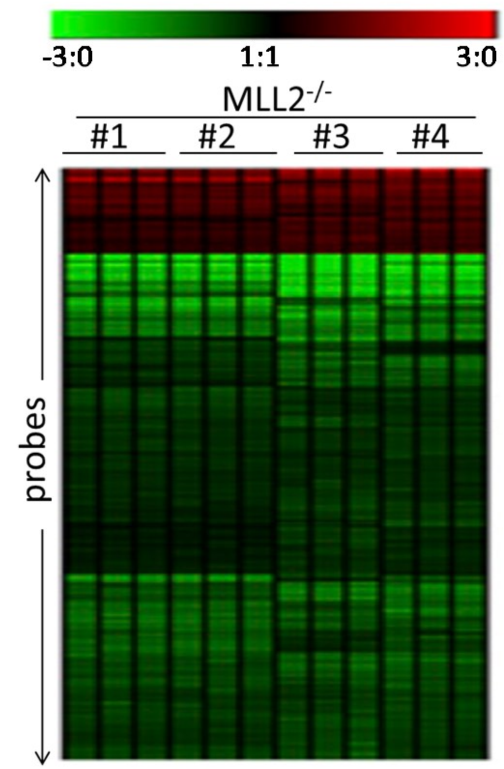


Fig 4. Expression profiling of parental and MLL2-knockout HCT116 cells. Unsupervised hierarchical clustering for probes with average linkage was performed to reveal differential expression genes in MLL2−/− clones (1–4) in comparison with parental cells. Triplicates for each clone were included and compared with the average expression value of parental cells. Red denotes relative overexpression, and green denotes relative reduced expression level. The exact levels of change for these probes are shown in Supplementary Dataset 1.

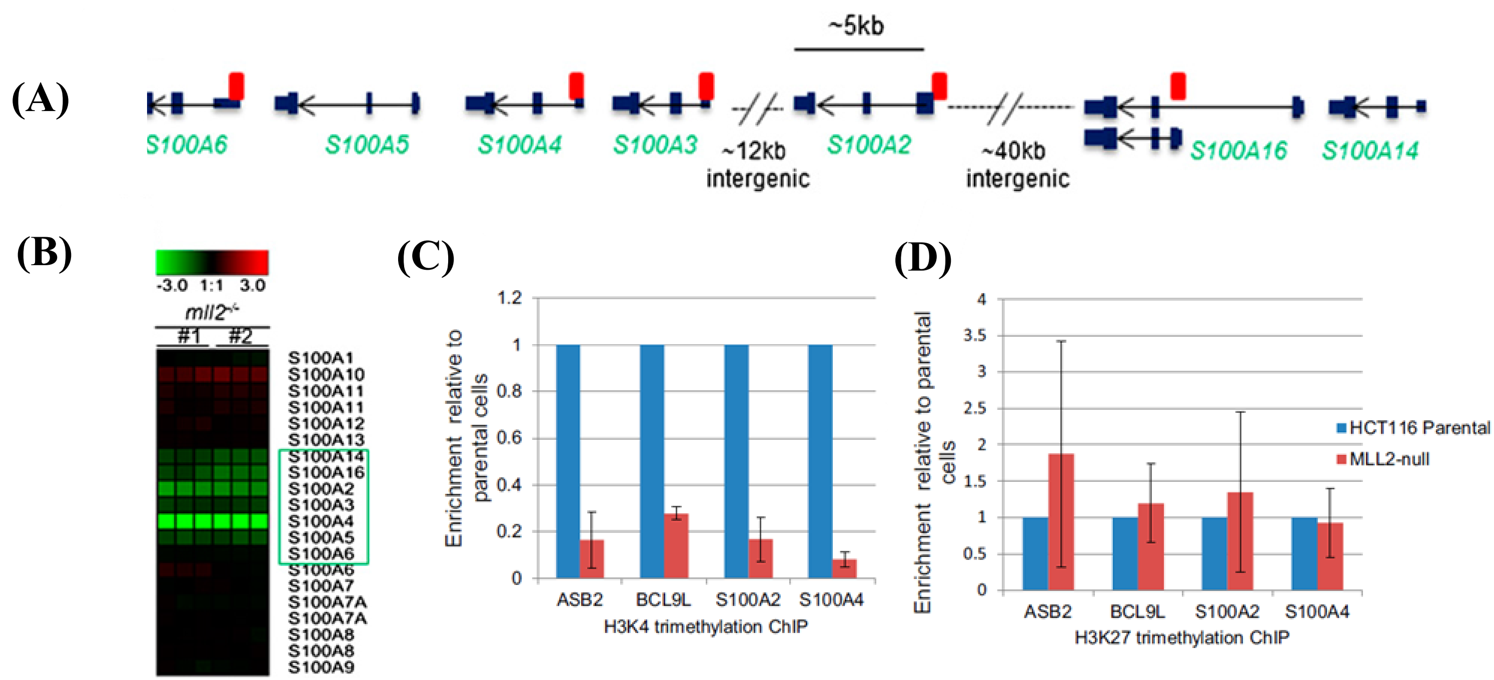


Fig 5. MLL2 binding to the S100A gene cluster. (A) Schematics of the S100A gene cluster region on chromosome 1q21.3. MLL2 bindings within this region (as identified in our previous study, citation) are marked (red rectangles). (B) Microarray profiling shows reduced expression of the clustered S100A genes in MLL2 $^{-/-}$ cells, in comparison with parental cells. (C and D) Reduced H3K4 trimethylation in MLL2 binding loci in MLL2$^{-/-}$ cells. ChIP coupled with quantitative PCR revealed reduced H3K4 trimethylation (C) and minor or no change in H3K27 trimethylation (D) in selected loci bound by MLL2 in MLL2$^{-/-}$ cells. Four genes were included for analysis. Results were from three independent ChIP experiments in an MLL2$^{-/-}$ HCT116 clone.

3.4 MLL2 Regulates Gene Expression of Multiple Signaling Pathways

To further gain an integrative view of the functional consequence of MLL2 in the context of cellular signaling pathways, we selected the genes whose expression was >50% down-regulated by MLL2 loss of function and subjected them to IPA canonical pathway analysis (Supplementary Dataset 1). Analysis of pathways that may be regulated by MLL2 revealed the potential effects of MLL2 on a broad range of cellular processes. Among those signaling pathways that were found to be significantly related were cAMP-mediated signaling and retinoic acid receptor (RXR) signaling (Table 3). Our data also revealed hepatic cholestasis pathway and B-cell development pathway, consistent with previous studies [11, 17, 18, 39]

3.5 MLL2 Regulates the Expression of the Retinoic Acid-Responsive Gene ASB2.

We next sought to validate the functional role of MLL2 in regulating the transcription of the genes we identified. The pathway analysis revealed that one of the interesting categories of genes was those that respond to nuclear hormones. Interestingly, one of the pathways overrepresented in both MLL2-bound and MLL2-responsive gene sets was retinoic acid signaling. One of the genes that were linked to the MLL2 binding loci was ASB2 [31], whose expression was previously found to be induced by retinoic acid in leukemia cells [40, 41]. This gene was expressed at a relatively low level in parental HCT116 cells, and quantitative analysis of the ASB2 transcript showed that its expression was further reduced in the MLL2$^{-/-}$ cell lines (Fig. 6A). To verify the role of the MLL2 complex in mediating the retinoic acid response, we tested whether HCT116 cells responded to retinoic acid transcriptionally using ASB2 as a marker gene. Treatment of parental HCT116 cells with retinoic acid induced robust ASB2 expression (Fig. 6B). Furthermore, whereas retinoic acid treatment also led to an increased ASB2 transcript level over a very low basal level in the MLL2$^{-/-}$ cells, the expression level of ASB2 was significantly lower than the level seen in the MLL2-intact parental cells upon retinoic acid treatment (Fig. 6B), indicating that MLL2 involvement accounted for the majority of increased ASB2 expression in response to retinoic acid. Collectively, these results validated the roles of MLL2 uncovered by gene expression studies, and suggest an extensive involvement of MLL2 in a variety of cellular signaling pathways.

Table 3. Ingenuity Pathway Analysis of genes down-regulated by MLL2 loss of function.

Pathway	*P* value	Gene list
cAMP-mediated signaling	0.0023	PDE12, PKIA, PKIB, RPS6KA1, PPP3CA
LPS/IL-1–mediated inhibition of RXR function	0.0024	FABP6, ABCC2, ABCC3, ACOX2, IL-18
Hepatic cholestasis	0.0032	ABCC2, ABCC3, FABP6, IL-18
FXR/RAR activation	0.0062	ABCC2, FABP6, IL-18
B-cell development	0.01	IL7, IL7R
Cardiac β-adrenergic signaling	0.0199	PDE12, PKIA, PKIB
Role of JAK1 and JAK3 in Yc cytokine signaling	0.0299	IL7, IL7R
PXR/RXR activation	0.0354	ABCC2, ABCC3
WNT/β-catenin signaling	0.0368	GJA1, SOX4, SOX9
Embryonic stem cell differentiation into cardiac lineages	0.0427	ISL1
HER-2 signaling in breast cancer	0.0433	ITGB4, NRG1

cAMP, cyclic adenosine monophosphate; FXR, farnesoid X receptor; LPS, lipopolysaccharide; RXR, retinoid X receptor; PXR, pregnane X receptor; RAR, retinoic acid receptor.

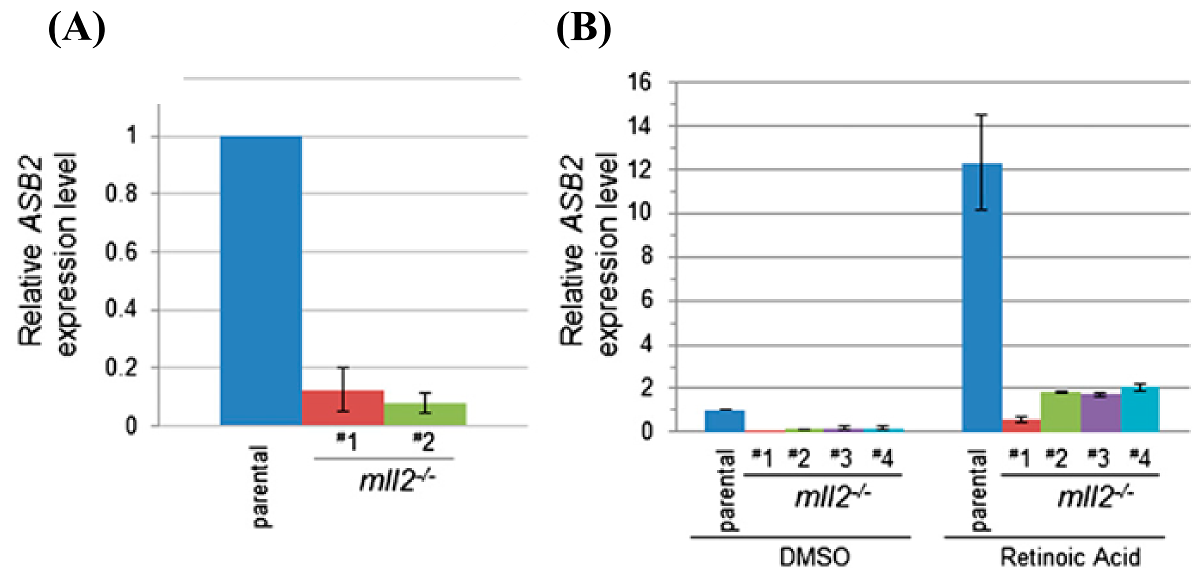


Fig 6. MLL2 regulates the expression of retinoic acid target genes. (A) Reduced expression of ASB2 (a retinoic acid response gene) in MLL2−/− cells, as determined by RT-qPCR. (B) Attenuated ASB2 gene expression in response to retinoic acid treatment in MLL2−/− cells. Four MLL2−/− lines derived from two different MLL2+/− progenitor lines were treated with DMSO or with 1 μM retinoic acid for 48 h. Quantitative RT-PCR was performed to determine the relative expression level of ASB2 genes. Error bars stand for standard deviation from three independent experiments.

4. Discussions

In this study, we generated MLL2 knockout cell lines for identification of genes whose expression was affected by MLL2 loss of function. The MLL2 deficient cells display slower growth and multinuclear morphology, which indicate the MLL2 function in cell cycle and/or cell senescence. A further examination such as cell cycle progression assay and senescence assay will be required to confirm our result. In addition to the biological finding, our gene expression analysis revealed a list of genes that are regulated by the MLL2 complex. Pathway analysis showed that the MLL2 complex plays roles in multiple signaling pathways and cellular processes, including cAMP signaling pathway, and retinoic acid signaling pathway. By comparing with our previous result of MLL2 binding loci [31], a subset of genes that were bound by MLL2 displays significant lower expression level in MLL2-null cells. Among those genes, we notice that MLL2 was associated with and likely directly regulated by the S100A family of genes, which encode proteins containing EF-hand calcium-binding motifs that regulate intracellular calcium signaling and participate in a number of cellular processes [42]. In particular, S100A2 was identified as a putative tumor suppressor [43, 44], possibly through regulating the p53 pathway [45]. The regulation of the S100A gene cluster by the MLL2 complex reveals an additional gene cluster that is transcriptionally co-regulated by an MLL-family epigenetic regulator.

Our findings provide evidence to expand previously established links between H3K4 methyltransferases and other pathways, and suggest that the roles of MLL2 may be extensive. Nuclear hormone receptors are among those at the top of the list in the transcription factor and pathway analysis from our gene expression profiling and binding sites of MLL2. In addition, among the most interesting discoveries are the signaling pathways that were not previously known to be associated with MLL2 or MLL3 complexes, such as cAMP signaling. These findings, and those from previous work, provide insight that may inform the future search for mechanisms underlying the role of MLL2 in pathogenesis: (i) It is possible that one or more of these currently described pathways contributes to MLL2 mutation-mediated tumorigenesis in a cell type specific manner; (ii) comparing gene expression profiles (MLL2 wild-type versus mutant) in different cellular contexts may suggest the primacy of these pathways in tumors bearing MLL2 pathway alterations; and (iii) gene expression signatures characteristic of MLL2 mutations may be exploited for tumor classification.

In summary, the present study advances our understanding of MLL2 in the following ways: (i) it identified various downstream pathways that are regulated by MLL2, establishing a basis for studying the mechanism underlying the role of MLL2 in development and disease; (ii) it uncovered the regulatory role of MLL2 in the transcription of clustered S100A-family genes that encode for a group of broadly distributed yet understudied proteins; (iii) it further established the extensive role of MLL2 in nuclear hormone signaling and provided a rationale for future studies on the link between nuclear hormone signaling and diseases associated with MLL2 alterations; and finally, (iv) our work further validated the somatic recombination-based approach for studying massive proteins, such as MLL2, for which overexpression is a challenging task. The findings and approaches undertaken here have laid the foundation for further understanding the function of MLL2 and have implications for future research on the rapidly accumulating list of epigenetic regulator genes that have been found to be altered in cancers and other diseases.

Supplementary Dataset 1

Microarray gene expression profiling analysis. List of genes that were down-regulated (<70% of parental cells) or up-regulated (>160% of parental cells) in $MLL2^{-/-}$ clone #1 and #2 and displayed significant change in #3 and #4.

Affymetrix Probe Set ID	gene description	gene symbol	expression level relative to parental (log2)	relative to parental expression (average of *MLL2*$^{-/-}$ #1 and #2)[a]	q-value (*MLL2*$^{-/-}$ line #1)[b]	q-value (*MLL2*$^{-/-}$ line #2)[b]	expression level relative to parental (log2)	relative to parental expression (average of *MLL2*$^{-/-}$ #3 and #4)[a]	q-value (*MLL2*$^{-/-}$ line #3)[b]	q-value (*MLL2*$^{-/-}$ line #4)[b]	Identified by ChIP	Used for IPA for generating Table 3 data[d]
213110_s_at	collagen, type IV, alpha 5	COL4A5	-5.62	0.02	<0.0001	<0.0001	-4.54	0.04	<0.0001	<0.0001	No	Yes
201667_at	gap junction protein, alpha 1, 43kDa	GJA1	-4.74	0.04	<0.0001	<0.0001	-3.88	0.07	<0.0001	<0.0001	No	Yes
226415_at	vesicle amine transport protein 1 homolog (T. californica)-like	VAT1L	-4.53	0.04	<0.0001	<0.0001	-3.14	0.11	<0.0001	<0.0001	No	Yes
206343_s_at	neuregulin 1	NRG1	-4.4	0.05	<0.0001	<0.0001	-1.34	0.4	<0.0001	<0.0001	No	Yes
1561691_at	hypothetical LOC285735	LOC285735	-4.23	0.05	<0.0001	<0.0001	-3.37	0.1	<0.0001	<0.0001	No	Yes
206504_at	cytochrome P450, family 24, subfamily A, polypeptide 1	CYP24A1	-3.69	0.08	<0.0001	<0.0001	-2.4	0.19	<0.0001	<0.0001	No	Yes
203186_s_at	S100 calcium binding protein A4	S100A4	-3.67	0.08	<0.0001	<0.0001	-3	0.13	<0.0001	<0.0001	No	Yes
206785_s_at	killer cell lectin-like receptor subfamily C, member 1 /// killer cell lectin-like receptor subfamily C, member 2	KLRC1 /// KLRC2	-3.33	0.1	<0.0001	<0.0001	-2.23	0.21	<0.0001	<0.0001	No	Yes
201426_s_at	vimentin	VIM	-3.21	0.11	<0.0001	<0.0001	-3.78	0.07	<0.0001	<0.0001	No	Yes
203453_at	sodium channel, nonvoltage-gated 1 alpha	SCNN1A	-3.15	0.11	<0.0001	<0.0001	-2.48	0.18	<0.0001	<0.0001	Yes	Yes
200665_s_at	secreted protein, acidic, cysteine-rich (osteonectin)	SPARC	-3.13	0.11	<0.0001	<0.0001	-2.54	0.17	<0.0001	<0.0001	No	Yes
207723_s_at	killer cell lectin-like receptor subfamily C, member 3	KLRC3	-3.02	0.12	<0.0001	<0.0001	-1.29	0.41	<0.0001	<0.0001	No	Yes
202935_s_at	SRY (sex determining region Y)-box 9	SOX9	-2.85	0.14	<0.0001	<0.0001	-1.89	0.27	<0.0001	<0.0001	No	Yes

204612_at	protein kinase (cAMP-dependent, catalytic) inhibitor alpha	PKIA	-2.81	0.14	<0.0001	<0.0001	-2.36	0.19	<0.0001	<0.0001	No	Yes
227452_at	hypothetical LOC100499467	LOC100499 467	-2.75	0.15	<0.0001	<0.0001	-1.82	0.28	<0.0001	<0.0001	No	Yes
219631_at	low density lipoprotein receptor-related protein 12	LRP12	-2.72	0.15	<0.0001	<0.0001	-2.89	0.13	<0.0001	<0.0001	No	Yes
223435_s_at	protocadherin alpha 1 /// protocadherin alpha 10 /// protocadherin alpha 11 /// protocadherin alpha 12 /// protocadherin alpha 13 /// protocadherin alpha 2 /// protocadherin alpha 3 /// protocadherin alpha 4 /// protocadherin alpha 5 /// protocadherin alpha 6 /// protocadherin alpha 7 /// protocadherin alpha 8 /// protocadherin alpha 9 /// protocadherin alpha subfamily C, 1 /// protocadherin alpha subfamily C, 2		-2.62	0.16	<0.0001	<0.0001	-1.98	0.25	<0.0001	<0.0001	No	Yes
240228_at	CUB and Sushi multiple domains 3	CSMD3	-2.61	0.16	<0.0001	<0.0001	-2.56	0.17	<0.0001	<0.0001	No	Yes
226864_at	protein kinase (cAMP-dependent, catalytic) inhibitor alpha	PKIA	-2.5	0.18	<0.0001	<0.0001	-1.73	0.3	<0.0001	<0.0001	No	Yes
205229_s_at	coagulation factor C homolog, cochlin (Limulus polyphemus)	COCH	-2.48	0.18	<0.0001	<0.0001	-1.24	0.42	<0.0001	<0.0001	No	Yes
202936_s_at	SRY (sex determining region Y)-box 9	SOX9	-2.48	0.18	<0.0001	<0.0001	-1.64	0.32	<0.0001	<0.0001	No	Yes
213992_at	collagen, type IV, alpha 6	COL4A6	-2.4	0.19	<0.0001	<0.0001	-1.52	0.35	<0.0001	<0.0001	No	Yes
206155_at	ATP-binding cassette, sub-family C (CFTR/MRP), member 2	ABCC2	-2.3	0.2	<0.0001	<0.0001	-2.6	0.16	<0.0001	<0.0001	Yes	Yes
221577_x_at	growth differentiation factor 15	GDF15	-2.28	0.21	<0.0001	<0.0001	-0.96	0.51	<0.0001	<0.0001	No	Yes

242873_at	---	---	-2.18	0.22	<0.0001	<0.0001	-1.18	0.44	<0.0001	<0.0001	No	Yes
201288_at	Rho GDP dissociation inhibitor (GDI) beta	ARHGDIB	-2.13	0.23	<0.0001	<0.0001	-3.06	0.12	<0.0001	<0.0001	No	Yes
226218_at	interleukin 7 receptor	IL7R	-2.09	0.23	<0.0001	<0.0001	-1.74	0.3	<0.0001	<0.0001	No	Yes
219710_at	SH3 domain and tetratricopeptide repeats 2	SH3TC2	-1.93	0.26	<0.0001	<0.0001	-1.42	0.37	<0.0001	<0.0001	Yes	Yes
219836_at	zinc finger, BED-type containing 2	ZBED2	-1.88	0.27	<0.0001	<0.0001	-0.39	0.76	0.0031	0.0011	No	Yes
201416_at	SRY (sex determining region Y)-box 4	SOX4	-1.81	0.29	<0.0001	<0.0001	-1.67	0.31	<0.0001	<0.0001	No	Yes
210445_at	fatty acid binding protein 6, ileal	FABP6	-1.8	0.29	<0.0001	<0.0001	-2.38	0.19	<0.0001	<0.0001	No	Yes
205364_at	acyl-CoA oxidase 2, branched chain	ACOX2	-1.77	0.29	<0.0001	<0.0001	-0.52	0.7	<0.0001	0.0011	No	Yes
209114_at	tetraspanin 1	TSPAN1	-1.77	0.29	<0.0001	<0.0001	-1.4	0.38	<0.0001	<0.0001	Yes	Yes
207426_s_at	tumor necrosis factor (ligand) superfamily, member 4	TNFSF4	-1.76	0.3	<0.0001	<0.0001	-1.32	0.4	<0.0001	<0.0001	No	Yes
204971_at	cystatin A (stefin A)	CSTA	-1.73	0.3	<0.0001	<0.0001	-1.98	0.25	<0.0001	<0.0001	No	Yes
201163_s_at	insulin-like growth factor binding protein 7	IGFBP7	-1.7	0.31	<0.0001	<0.0001	-1.01	0.5	<0.0001	<0.0001	No	Yes
243871_at	---	---	-1.69	0.31	<0.0001	<0.0001	-1.6	0.33	<0.0001	<0.0001	No	Yes
225996_at	LON peptidase N-terminal domain and ring finger 2	LONRF2	-1.66	0.32	<0.0001	<0.0001	-0.49	0.71	<0.0001	0.0021	No	Yes
201417_at	SRY (sex determining region Y)-box 4	SOX4	-1.65	0.32	<0.0001	<0.0001	-1.17	0.44	<0.0001	<0.0001	No	Yes
207534_at	melanoma antigen family B, 1	MAGEB1	-1.61	0.33	<0.0001	<0.0001	-1.52	0.35	<0.0001	<0.0001	No	Yes
231120_x_at	protein kinase (cAMP-dependent, catalytic) inhibitor beta	PKIB	-1.6	0.33	<0.0001	<0.0001	-2.43	0.19	<0.0001	<0.0001	No	Yes
206104_at	ISL LIM homeobox 1	ISL1	-1.58	0.33	<0.0001	<0.0001	-1.11	0.46	<0.0001	<0.0001	No	Yes
204268_at	S100 calcium binding protein A2	S100A2	-1.57	0.34	<0.0001	<0.0001	-1.11	0.46	<0.0001	<0.0001	Yes	Yes
1552334_at	TRIO and F-actin binding protein	TRIOBP	-1.57	0.34	<0.0001	<0.0001	-0.97	0.51	<0.0001	<0.0001	No	Yes
203379_at	ribosomal protein S6 kinase, 90kDa, polypeptide 1	RPS6KA1	-1.55	0.34	<0.0001	<0.0001	-1.53	0.35	<0.0001	<0.0001	No	Yes
205513_at	transcobalamin I (vitamin B12 binding protein, R binder family)	TCN1	-1.54	0.34	<0.0001	<0.0001	-1.47	0.36	<0.0001	<0.0001	No	Yes
1555812_a_at	Rho GDP dissociation inhibitor (GDI) beta	ARHGDIB	-1.51	0.35	<0.0001	<0.0001	-1.92	0.26	<0.0001	<0.0001	No	Yes
203964_at	N-myc (and STAT) interactor	NMI	-1.51	0.35	<0.0001	<0.0001	-1.55	0.34	<0.0001	<0.0001	No	Yes

226803_at	chromatin modifying protein 4C	CHMP4C	-1.51	0.35	<0.0001	<0.0001	-1.65	0.32	<0.0001	<0.0001	No	Yes
213668_s_at	SRY (sex determining region Y)-box 4	SOX4	-1.51	0.35	<0.0001	<0.0001	-1.12	0.46	<0.0001	<0.0001	No	Yes
231325_at	unc-5 homolog D (C. elegans)	UNC5D	-1.49	0.36	<0.0001	<0.0001	-0.87	0.55	<0.0001	<0.0001	No	Yes
235521_at	homeobox A3	HOXA3	-1.49	0.36	<0.0001	<0.0001	-1.6	0.33	<0.0001	<0.0001	No	Yes
212070_at	G protein-coupled receptor 56	GPR56	-1.48	0.36	<0.0001	<0.0001	-0.91	0.53	<0.0001	<0.0001	No	Yes
223500_at	complexin 1	CPLX1	-1.43	0.37	<0.0001	<0.0001	-2.02	0.25	<0.0001	<0.0001	No	Yes
205399_at	doublecortin-like kinase 1	DCLK1	-1.42	0.37	<0.0001	<0.0001	0.98	1.98	<0.0001	<0.0001	No	Yes
1552332_at	TRIO and F-actin binding protein	TRIOBP	-1.41	0.38	<0.0001	<0.0001	-0.63	0.65	<0.0001	<0.0001	No	Yes
225165_at	protein phosphatase 1, regulatory (inhibitor) subunit 1B	PPP1R1B	-1.4	0.38	<0.0001	<0.0001	-0.81	0.57	<0.0001	<0.0001	No	Yes
1554242_a_at	coagulation factor C homolog, cochlin (Limulus polyphemus)	COCH	-1.4	0.38	<0.0001	<0.0001	-1.04	0.49	<0.0001	<0.0001	No	Yes
204990_s_at	integrin, beta 4	ITGB4	-1.36	0.39	<0.0001	<0.0001	-0.82	0.57	<0.0001	<0.0001	No	Yes
214079_at	dehydrogenase/reductase (SDR family) member 2	DHRS2	-1.35	0.39	<0.0001	<0.0001	-1.54	0.34	<0.0001	<0.0001	No	Yes
225968_at	prickle homolog 2 (Drosophila)	PRICKLE2	-1.34	0.39	<0.0001	<0.0001	-0.95	0.52	<0.0001	<0.0001	No	Yes
220310_at	tubulin, alpha-like 3	TUBAL3	-1.34	0.4	<0.0001	<0.0001	-0.97	0.51	<0.0001	<0.0001	No	Yes
209443_at	serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 5	SERPINA5	-1.33	0.4	<0.0001	<0.0001	-0.59	0.67	<0.0001	<0.0001	No	Yes
212444_at	---	---	-1.33	0.4	<0.0001	<0.0001	-0.45	0.73	<0.0001	<0.0001	No	Yes
236489_at	G protein-coupled receptor 110	GPR110	-1.3	0.4	<0.0001	<0.0001	-1.14	0.45	<0.0001	<0.0001	No	Yes
206295_at	interleukin 18 (interferon-gamma-inducing factor)	IL18	-1.28	0.41	<0.0001	<0.0001	-0.89	0.54	<0.0001	<0.0001	No	Yes
238689_at	G protein-coupled receptor 110	GPR110	-1.27	0.41	<0.0001	<0.0001	-1.3	0.41	<0.0001	<0.0001	No	Yes
204855_at	serpin peptidase inhibitor, clade B (ovalbumin), member 5	SERPINB5	-1.27	0.41	<0.0001	<0.0001	-1.28	0.41	<0.0001	<0.0001	No	Yes
211864_s_at	myoferlin	MYOF	-1.26	0.42	<0.0001	<0.0001	-0.71	0.61	<0.0001	<0.0001	Yes	Yes
209765_at	ADAM metallopeptidase domain 19	ADAM19	-1.25	0.42	<0.0001	<0.0001	-1.09	0.47	<0.0001	<0.0001	No	Yes
1555673_at	keratin associated protein 2-4-like	LOC730755	-1.25	0.42	<0.0001	<0.0001	-0.45	0.73	0.0006	<0.0001	No	Yes
222740_at	ATPase family, AAA domain containing 2	ATAD2	-1.25	0.42	<0.0001	<0.0001	-1.38	0.38	<0.0001	<0.0001	Yes	Yes
1554915_a_at	phosphodiesterase 12	PDE12	-1.24	0.42	<0.0001	<0.0001	-1.07	0.48	<0.0001	<0.0001	No	Yes

223551_at	protein kinase (cAMP-dependent, catalytic) inhibitor beta	PKIB	-1.24	0.42	<0.0001	<0.0001	-2.36	0.19	<0.0001	<0.0001	No	Yes
223786_at	carbohydrate (N-acetylglucosamine 6-O) sulfotransferase 6	CHST6	-1.23	0.43	<0.0001	<0.0001	-0.82	0.57	<0.0001	<0.0001	No	Yes
242005_at	hypothetical LOC100506377	LOC100506 377	-1.21	0.43	<0.0001	<0.0001	-1.47	0.36	<0.0001	<0.0001	No	Yes
228401_at	ATPase family, AAA domain containing 2	ATAD2	-1.2	0.44	<0.0001	<0.0001	-1	0.5	<0.0001	<0.0001	Yes	Yes
202429_s_at	protein phosphatase 3, catalytic subunit, alpha isozyme	PPP3CA	-1.18	0.44	<0.0001	<0.0001	-0.87	0.55	<0.0001	<0.0001	Yes	Yes
201798_s_at	myoferlin	MYOF	-1.18	0.44	<0.0001	<0.0001	-0.74	0.6	<0.0001	<0.0001	Yes	Yes
228640_at	protocadherin 7	PCDH7	-1.17	0.44	<0.0001	<0.0001	-0.84	0.56	<0.0001	<0.0001	No	Yes
218352_at	regulator of chromosome condensation (RCC1) and BTB (POZ) domain containing protein 1	RCBTB1	-1.14	0.45	<0.0001	<0.0001	-0.56	0.68	<0.0001	<0.0001	No	Yes
202457_s_at	protein phosphatase 3, catalytic subunit, alpha isozyme	PPP3CA	-1.12	0.46	<0.0001	<0.0001	-0.97	0.51	<0.0001	<0.0001	Yes	Yes
230831_at	FERM domain containing 5	FRMD5	-1.12	0.46	<0.0001	<0.0001	-0.95	0.52	<0.0001	<0.0001	No	Yes
203108_at	G protein-coupled receptor, family C, group 5, member A	GPRC5A	-1.12	0.46	<0.0001	<0.0001	-0.75	0.59	<0.0001	<0.0001	No	Yes
204194_at	BTB and CNC homology 1, basic leucine zipper transcription factor 1	BACH1	-1.11	0.46	<0.0001	<0.0001	-0.39	0.77	<0.0001	<0.0001	No	Yes
241367_at	testis expressed 19	TEX19	-1.11	0.46	<0.0001	<0.0001	-0.79	0.58	<0.0001	<0.0001	No	Yes

210674_s_at	protocadherin alpha 1 /// protocadherin alpha 10 /// protocadherin alpha 11 /// protocadherin alpha 12 /// protocadherin alpha 13 /// protocadherin alpha 2 /// protocadherin alpha 3 /// protocadherin alpha 4 /// protocadherin alpha 5 /// protocadherin alpha 6 /// protocadherin alpha 7 /// protocadherin alpha 8 /// protocadherin alpha 9 /// protocadherin alpha subfamily C, 1 /// protocadherin alpha subfamily C, 2	#VALUE!	-1.1	0.47	<0.0001	<0.0001	-0.65	0.64	<0.0001	<0.0001	No	Yes
223590_at	zinc finger protein 700	ZNF700	-1.1	0.47	<0.0001	<0.0001	-1.15	0.45	<0.0001	<0.0001	No	Yes
234219_at	---	---	-1.1	0.47	<0.0001	<0.0001	-0.96	0.51	<0.0001	<0.0001	No	Yes
235266_at	ATPase family, AAA domain containing 2	ATAD2	-1.09	0.47	<0.0001	<0.0001	-1.27	0.42	<0.0001	<0.0001	Yes	Yes
1557779_at	---	---	-1.09	0.47	<0.0001	<0.0001	-1.34	0.4	<0.0001	<0.0001	No	Yes
218782_s_at	ATPase family, AAA domain containing 2	ATAD2	-1.08	0.47	<0.0001	<0.0001	-1.3	0.4	<0.0001	<0.0001	Yes	Yes
208161_s_at	ATP-binding cassette, sub-family C (CFTR/MRP), member 3	ABCC3	-1.08	0.47	<0.0001	<0.0001	-1.27	0.41	<0.0001	<0.0001	Yes	Yes
209369_at	annexin A3	ANXA3	-1.06	0.48	<0.0001	<0.0001	-0.39	0.76	0.0006	<0.0001	No	Yes
206693_at	interleukin 7	IL7	-1.06	0.48	<0.0001	<0.0001	-1.12	0.46	<0.0001	<0.0001	No	Yes
206463_s_at	dehydrogenase/reductase (SDR family) member 2	DHRS2	-1.06	0.48	<0.0001	<0.0001	-1.23	0.43	<0.0001	<0.0001	No	Yes
202957_at	hematopoietic cell-specific Lyn substrate 1	HCLS1	-1.06	0.48	<0.0001	<0.0001	-0.61	0.65	<0.0001	<0.0001	No	Yes
235988_at	G protein-coupled receptor 110	GPR110	-1.05	0.48	<0.0001	<0.0001	-1.24	0.42	<0.0001	<0.0001	No	Yes
242127_at	---	---	-1.04	0.49	<0.0001	<0.0001	-0.88	0.54	<0.0001	<0.0001	No	Yes
226776_at	enhancer of yellow 2 homolog (Drosophila)	ENY2	-1.03	0.49	<0.0001	<0.0001	-0.63	0.65	<0.0001	0.0011	No	Yes
210276_s_at	nucleolar protein 12 /// TRIO and F-actin binding protein	NOL12 /// TRIOBP	-1.03	0.49	<0.0001	<0.0001	-1.03	0.49	<0.0001	<0.0001	No	Yes

214022_s_at	interferon induced transmembrane protein 1 (9-27)	IFITM1	-1.03	0.49	<0.0001	<0.0001	-0.84	0.56	<0.0001	<0.0001	No	Yes
230398_at	tensin 4	TNS4	-1.01	0.5	<0.0001	<0.0001	-0.32	0.8	0.0031	0.0039	Yes	Yes
215331_at	myosin, heavy chain 15	MYH15	-1	0.5	<0.0001	<0.0001	-0.68	0.62	<0.0001	<0.0001	No	Yes
227998_at	S100 calcium binding protein A16	S100A16	-0.99	0.5	0.0006	<0.0001	-1.32	0.4	<0.0001	<0.0001	No	No
205266_at	leukemia inhibitory factor (cholinergic differentiation factor)	LIF	-0.99	0.5	<0.0001	<0.0001	-0.57	0.67	<0.0001	<0.0001	No	No
219526_at	chromosome 14 open reading frame 169	C14orf169	-0.98	0.51	0.0006	<0.0001	-0.53	0.69	<0.0001	<0.0001	No	No
216210_x_at	TRIO and F-actin binding protein	TRIOBP	-0.98	0.51	<0.0001	<0.0001	-1.02	0.49	<0.0001	<0.0001	No	No
201860_s_at	plasminogen activator, tissue	PLAT	-0.97	0.51	<0.0001	<0.0001	-0.44	0.74	0.0031	<0.0001	No	No
208883_at	ubiquitin protein ligase E3 component n-recognin 5	UBR5	-0.96	0.52	<0.0001	<0.0001	-0.85	0.55	<0.0001	<0.0001	No	No
1552390_a_at	chromosome 8 open reading frame 47	C8orf47	-0.95	0.52	<0.0001	<0.0001	-1.41	0.38	<0.0001	<0.0001	No	No
218718_at	platelet derived growth factor C	PDGFC	-0.95	0.52	<0.0001	<0.0001	-1.49	0.36	<0.0001	<0.0001	No	No
224269_at	keratin associated protein 4-12	KRTAP4-12	-0.95	0.52	0.0006	0.0017	-0.78	0.58	<0.0001	<0.0001	No	No
229606_at	---	---	-0.94	0.52	<0.0001	<0.0001	-0.89	0.54	<0.0001	<0.0001	No	No
204011_at	sprouty homolog 2 (Drosophila)	SPRY2	-0.94	0.52	<0.0001	<0.0001	-1.81	0.29	<0.0001	<0.0001	No	No
210619_s_at	hyaluronoglucosaminidase 1	HYAL1	-0.93	0.52	<0.0001	<0.0001	-0.62	0.65	0.0069	<0.0001	Yes	No
203567_s_at	tripartite motif-containing 38	TRIM38	-0.92	0.53	<0.0001	<0.0001	-0.84	0.56	<0.0001	<0.0001	No	No
202425_x_at	protein phosphatase 3, catalytic subunit, alpha isozyme	PPP3CA	-0.92	0.53	<0.0001	<0.0001	-0.92	0.53	<0.0001	<0.0001	Yes	No
1554168_a_at	SH3-domain kinase binding protein 1	SH3KBP1	-0.91	0.53	<0.0001	<0.0001	-0.76	0.59	<0.0001	<0.0001	No	No
208882_s_at	ubiquitin protein ligase E3 component n-recognin 5	UBR5	-0.9	0.54	<0.0001	<0.0001	-0.96	0.51	<0.0001	<0.0001	No	No
1552389_at	chromosome 8 open reading frame 47	C8orf47	-0.9	0.54	<0.0001	<0.0001	-1.11	0.46	<0.0001	<0.0001	No	No
223672_at	SH3-domain GRB2-like (endophilin) interacting protein 1	SGIP1	-0.9	0.54	0.0009	0.0005	-1.17	0.44	<0.0001	<0.0001	No	No
218976_at	DnaJ (Hsp40) homolog, subfamily C, member 12	DNAJC12	-0.89	0.54	<0.0001	<0.0001	-0.84	0.56	<0.0001	<0.0001	No	No
39248_at	aquaporin 3 (Gill blood group)	AQP3	-0.89	0.54	<0.0001	<0.0001	-0.95	0.52	<0.0001	<0.0001	No	No

231839_at	phosphodiesterase 12	PDE12	-0.89	0.54	<0.0001	<0.0001	-0.79	0.58	<0.0001	<0.0001	No	No
202447_at	2,4-dienoyl CoA reductase 1, mitochondrial	DECR1	-0.89	0.54	<0.0001	<0.0001	-0.65	0.64	<0.0001	<0.0001	No	No
204823_at	neuron navigator 3	NAV3	-0.89	0.54	<0.0001	<0.0001	-1.94	0.26	<0.0001	<0.0001	No	No
231872_at	leucine rich repeat and coiled-coil domain containing 1	LRRCC1	-0.89	0.54	<0.0001	<0.0001	-0.69	0.62	<0.0001	<0.0001	No	No
236105_at	zinc finger and BTB domain containing 10	ZBTB10	-0.88	0.54	<0.0001	<0.0001	-0.63	0.65	<0.0001	0.0011	No	No
212094_at	paternally expressed 10	PEG10	-0.87	0.55	<0.0001	<0.0001	-0.88	0.54	<0.0001	<0.0001	No	No
236545_at	---	---	-0.87	0.55	0.0006	0.0005	-1.22	0.43	<0.0001	<0.0001	No	No
228496_s_at	Cysteine rich transmembrane BMP regulator 1 (chordin-like)	CRIM1	-0.87	0.55	<0.0001	<0.0001	-0.71	0.61	<0.0001	<0.0001	No	No
209457_at	dual specificity phosphatase 5	DUSP5	-0.86	0.55	<0.0001	<0.0001	-0.23	0.85	0.0372	0.0091	Yes	No
1552546_a_at	leucine zipper-EF-hand containing transmembrane protein 2	LETM2	-0.86	0.55	0.0006	<0.0001	-1.16	0.45	<0.0001	<0.0001	No	No
219487_at	Bardet-Biedl syndrome 10	BBS10	-0.86	0.55	<0.0001	<0.0001	-0.81	0.57	<0.0001	<0.0001	No	No
240239_at	zinc finger protein 566	ZNF566	-0.85	0.55	<0.0001	<0.0001	-0.63	0.65	<0.0001	<0.0001	No	No
210017_at	mucosa associated lymphoid tissue lymphoma translocation gene 1	MALT1	-0.85	0.55	<0.0001	<0.0001	-0.79	0.58	<0.0001	<0.0001	No	No
202887_s_at	DNA-damage-inducible transcript 4	DDIT4	-0.85	0.56	0.0006	<0.0001	-0.9	0.54	<0.0001	<0.0001	No	No
223082_at	SH3-domain kinase binding protein 1	SH3KBP1	-0.84	0.56	<0.0001	<0.0001	-0.77	0.59	<0.0001	<0.0001	No	No
202795_x_at	TRIO and F-actin binding protein	TRIOBP	-0.84	0.56	<0.0001	<0.0001	-0.93	0.52	<0.0001	<0.0001	No	No
226425_at	CAP-GLY domain containing linker protein family, member 4	CLIP4	-0.83	0.56	<0.0001	<0.0001	-0.64	0.64	<0.0001	<0.0001	No	No
224646_x_at	H19, imprinted maternally expressed transcript (non-protein coding)	H19	-0.83	0.56	<0.0001	<0.0001	-0.68	0.62	<0.0001	<0.0001	No	No
227094_at	dehydrogenase E1 and transketolase domain containing 1	DHTKD1	-0.82	0.57	0.0006	<0.0001	-0.8	0.58	<0.0001	<0.0001	No	No
217109_at	mucin 4, cell surface associated	MUC4	-0.82	0.57	0.0006	<0.0001	-0.77	0.59	<0.0001	0.0011	No	No

201474_s_at	integrin, alpha 3 (antigen CD49C, alpha 3 subunit of VLA-3 receptor)	ITGA3	-0.82	0.57	<0.0001	<0.0001	-1	0.5	<0.0001	<0.0001	Yes	No
211603_s_at	ets variant 4	ETV4	-0.82	0.57	<0.0001	<0.0001	-0.48	0.72	<0.0001	<0.0001	No	No
200931_s_at	vinculin	VCL	-0.81	0.57	<0.0001	<0.0001	-0.6	0.66	<0.0001	<0.0001	No	No
225902_at	peptidylprolyl isomerase G (cyclophilin G)	PPIG	-0.81	0.57	<0.0001	<0.0001	-0.67	0.63	<0.0001	<0.0001	No	No
228562_at	zinc finger and BTB domain containing 10	ZBTB10	-0.81	0.57	<0.0001	<0.0001	-0.84	0.56	<0.0001	<0.0001	No	No
202381_at	ADAM metallopeptidase domain 9	ADAM9	-0.8	0.57	<0.0001	<0.0001	-0.43	0.74	<0.0001	0.0011	Yes	No
209641_s_at	ATP-binding cassette, sub-family C (CFTR/MRP), member 3	ABCC3	-0.8	0.57	0.0006	<0.0001	-0.83	0.56	<0.0001	<0.0001	Yes	No
203961_at	nebulette	NEBL	-0.8	0.58	<0.0001	<0.0001	-0.57	0.67	<0.0001	<0.0001	No	No
222421_at	ubiquitin-conjugating enzyme E2H (UBC8 homolog, yeast)	UBE2H	-0.8	0.58	<0.0001	<0.0001	-0.58	0.67	<0.0001	<0.0001	Yes	No
202907_s_at	nibrin	NBN	-0.79	0.58	<0.0001	<0.0001	-0.59	0.67	<0.0001	<0.0001	No	No
218677_at	S100 calcium binding protein A14	S100A14	-0.79	0.58	<0.0001	<0.0001	-0.81	0.57	<0.0001	<0.0001	No	No
212461_at	antizyme inhibitor 1	AZIN1	-0.79	0.58	<0.0001	<0.0001	-0.64	0.64	<0.0001	<0.0001	No	No
201078_at	transmembrane 9 superfamily member 2	TM9SF2	-0.79	0.58	<0.0001	<0.0001	-0.69	0.62	<0.0001	<0.0001	No	No
202293_at	stromal antigen 1	STAG1	-0.79	0.58	<0.0001	<0.0001	-0.73	0.6	<0.0001	<0.0001	No	No
223595_at	transmembrane protein 133	TMEM133	-0.79	0.58	<0.0001	<0.0001	-0.88	0.54	<0.0001	<0.0001	No	No
212312_at	BCL2-like 1	BCL2L1	-0.78	0.58	<0.0001	<0.0001	-0.36	0.78	0.0006	<0.0001	Yes	No
201418_s_at	SRY (sex determining region Y)-box 4	SOX4	-0.78	0.58	<0.0001	<0.0001	-0.55	0.68	<0.0001	<0.0001	No	No
220254_at	low density lipoprotein receptor-related protein 12	LRP12	-0.78	0.58	0.0006	0.0017	-0.81	0.57	<0.0001	<0.0001	No	No
207763_at	S100 calcium binding protein A5	S100A5	-0.78	0.58	<0.0001	<0.0001	-0.77	0.59	<0.0001	<0.0001	No	No
232151_at	metastasis associated in colon cancer 1	MACC1	-0.78	0.58	0.0009	0.0005	-0.49	0.71	<0.0001	<0.0001	No	No
209090_s_at	SH3-domain GRB2-like endophilin B1	SH3GLB1	-0.78	0.58	<0.0001	<0.0001	-0.4	0.76	<0.0001	<0.0001	No	No
215000_s_at	fasciculation and elongation protein zeta 2 (zygin II)	FEZ2	-0.78	0.58	<0.0001	<0.0001	-0.57	0.67	<0.0001	<0.0001	No	No
231784_s_at	DDB1 and CUL4 associated factor 13	DCAF13	-0.77	0.58	<0.0001	<0.0001	-0.63	0.65	<0.0001	<0.0001	No	No
206254_at	epidermal growth factor	EGF	-0.77	0.59	0.0006	<0.0001	-0.51	0.7	<0.0001	0.0011	No	No
226086_at	synaptotagmin XIII	SYT13	-0.77	0.59	<0.0001	<0.0001	-0.5	0.71	<0.0001	0.0011	No	No

213194_at	roundabout, axon guidance receptor, homolog 1 (Drosophila)	ROBO1	-0.76	0.59	<0.0001	<0.0001	-0.84	0.56	<0.0001	<0.0001	No	No
225972_at	transmembrane protein 64	TMEM64	-0.75	0.59	<0.0001	<0.0001	-0.32	0.8	0.0031	0.0011	No	No
203962_s_at	nebulette	NEBL	-0.75	0.59	<0.0001	<0.0001	-0.55	0.68	<0.0001	<0.0001	No	No
214164_x_at	carbonic anhydrase XII	CA12	-0.75	0.6	0.0006	0.0017	-0.54	0.69	<0.0001	<0.0001	No	No
242871_at	progestin and adipoQ receptor family member V	PAQR5	-0.75	0.6	<0.0001	<0.0001	-0.7	0.62	<0.0001	<0.0001	No	No
205428_s_at	calbindin 2	CALB2	-0.74	0.6	0.0006	<0.0001	-0.63	0.65	<0.0001	<0.0001	No	No
204041_at	monoamine oxidase B	MAOB	-0.74	0.6	<0.0001	<0.0001	-0.74	0.6	<0.0001	<0.0001	No	No
236075_s_at	hypothetical LOC100506676	LOC100506 676	-0.73	0.6	<0.0001	<0.0001	-0.66	0.63	<0.0001	<0.0001	No	No
238423_at	synaptotagmin-like 3	SYTL3	-0.73	0.6	0.0049	<0.0001	-0.16	0.89	0.0848	0.058	No	No
239093_at	dihydrodipicolinate synthase-like, mitochondrial	DHDPSL	-0.73	0.6	<0.0001	<0.0001	-0.48	0.72	<0.0001	0.0011	No	No
207199_at	telomerase reverse transcriptase	TERT	-0.72	0.61	<0.0001	<0.0001	-0.86	0.55	<0.0001	<0.0001	No	No
210868_s_at	ELOVL family member 6, elongation of long chain fatty acids (FEN1/Elo2, SUR4/Elo3-like, yeast)	ELOVL6	-0.71	0.61	<0.0001	<0.0001	-0.64	0.64	<0.0001	<0.0001	No	No
203789_s_at	sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3C	SEMA3C	-0.71	0.61	0.0049	<0.0001	-1.29	0.41	<0.0001	<0.0001	No	No
210592_s_at	spermidine/spermine N1-acetyltransferase 1	SAT1	-0.71	0.61	0.0006	<0.0001	-0.68	0.62	<0.0001	<0.0001	No	No
227491_at	ELOVL family member 6, elongation of long chain fatty acids (FEN1/Elo2, SUR4/Elo3-like, yeast)	ELOVL6	-0.71	0.61	<0.0001	<0.0001	-0.37	0.78	<0.0001	<0.0001	No	No
222719_s_at	platelet derived growth factor C	PDGFC	-0.71	0.61	<0.0001	<0.0001	-1.1	0.46	<0.0001	<0.0001	No	No
219123_at	zinc finger protein 232	ZNF232	-0.71	0.61	<0.0001	<0.0001	-0.59	0.67	<0.0001	<0.0001	No	No
207358_x_at	microtubule-actin crosslinking factor 1	MACF1	-0.71	0.61	<0.0001	<0.0001	-0.67	0.63	<0.0001	<0.0001	No	No
208884_s_at	ubiquitin protein ligase E3 component n-recognin 5	UBR5	-0.7	0.61	<0.0001	<0.0001	-0.87	0.55	<0.0001	<0.0001	No	No

204401_at	potassium intermediate/small conductance calcium-activated channel, subfamily N, member 4	KCNN4	-0.7	0.61	<0.0001	<0.0001	-1.03	0.49	<0.0001	<0.0001	Yes	No
201691_s_at	tumor protein D52	TPD52	-0.7	0.62	0.0006	<0.0001	-0.43	0.74	<0.0001	0.0011	No	No
219312_s_at	zinc finger and BTB domain containing 10	ZBTB10	-0.7	0.62	<0.0001	0.0017	-0.85	0.55	<0.0001	<0.0001	No	No
203998_s_at	synaptotagmin I	SYT1	-0.7	0.62	0.0006	<0.0001	-1.29	0.41	<0.0001	<0.0001	No	No
217299_s_at	nibrin	NBN	-0.7	0.62	<0.0001	<0.0001	-0.52	0.7	<0.0001	<0.0001	No	No
209365_s_at	extracellular matrix protein 1	ECM1	-0.7	0.62	<0.0001	<0.0001	-0.38	0.77	<0.0001	<0.0001	No	No
239288_at	TRAF2 and NCK interacting kinase	TNIK	-0.69	0.62	<0.0001	<0.0001	-1.57	0.34	<0.0001	<0.0001	No	No
243818_at	surfactant associated 1 (pseudogene)	SFTA1P	-0.69	0.62	<0.0001	<0.0001	-2.55	0.17	<0.0001	<0.0001	No	No
213107_at	TRAF2 and NCK interacting kinase	TNIK	-0.68	0.62	<0.0001	<0.0001	-1.18	0.44	<0.0001	<0.0001	No	No
211828_s_at	TRAF2 and NCK interacting kinase	TNIK	-0.68	0.62	0.0006	<0.0001	-1.31	0.4	<0.0001	<0.0001	No	No
223256_at	G2/M-phase specific E3 ubiquitin protein ligase	G2E3	-0.68	0.62	<0.0001	<0.0001	-0.61	0.65	<0.0001	<0.0001	No	No
204068_at	serine/threonine kinase 3	STK3	-0.68	0.63	<0.0001	<0.0001	-0.27	0.83	0.016	0.0091	No	No
211538_s_at	heat shock 70kDa protein 2	HSPA2	-0.68	0.63	0.0006	<0.0001	-0.63	0.64	<0.0001	<0.0001	No	No
204226_at	staufen, RNA binding protein, homolog 2 (Drosophila)	STAU2	-0.67	0.63	<0.0001	<0.0001	-0.32	0.8	0.0031	0.0021	No	No
208995_s_at	peptidylprolyl isomerase G (cyclophilin G)	PPIG	-0.67	0.63	0.0006	<0.0001	-0.59	0.67	<0.0001	<0.0001	No	No
209270_at	laminin, beta 3	LAMB3	-0.67	0.63	0.0006	<0.0001	-0.64	0.64	<0.0001	<0.0001	Yes	No
238229_at	transmembrane protein 67	TMEM67	-0.67	0.63	<0.0001	<0.0001	-0.52	0.7	<0.0001	<0.0001	No	No
225342_at	adenylate kinase 4	AK4	-0.66	0.63	<0.0001	<0.0001	-0.43	0.74	<0.0001	0.0011	No	No
202551_s_at	cysteine rich transmembrane BMP regulator 1 (chordin-like)	CRIM1	-0.66	0.63	<0.0001	<0.0001	-0.52	0.7	<0.0001	<0.0001	No	No
203790_s_at	heat-responsive protein 12	HRSP12	-0.66	0.63	<0.0001	<0.0001	-0.59	0.66	<0.0001	<0.0001	No	No
209481_at	SNF related kinase	SNRK	-0.65	0.64	<0.0001	<0.0001	-0.71	0.61	<0.0001	<0.0001	No	No
226124_at	zinc finger protein 90 homolog (mouse)	ZFP90	-0.65	0.64	0.0006	<0.0001	-0.94	0.52	<0.0001	<0.0001	No	No
237034_at	---	---	-0.65	0.64	<0.0001	<0.0001	-1.17	0.44	<0.0001	<0.0001	No	No
210136_at	myelin basic protein	MBP	-0.65	0.64	0.0006	<0.0001	-0.91	0.53	<0.0001	<0.0001	No	No
207768_at	early growth response 4	EGR4	-0.65	0.64	0.0009	0.0005	-1	0.5	<0.0001	<0.0001	No	No
1560587_s_at	peroxiredoxin 5	PRDX5	-0.65	0.64	<0.0001	<0.0001	-0.64	0.64	<0.0001	<0.0001	No	No
212638_s_at	WW domain containing E3 ubiquitin protein ligase 1	WWP1	-0.65	0.64	0.0006	<0.0001	-0.49	0.71	<0.0001	<0.0001	No	No

203449_s_at	telomeric repeat binding factor (NIMA-interacting) 1	TERF1	-0.65	0.64	<0.0001	<0.0001	-0.37	0.78	0.0031	0.0039	No	No
213109_at	TRAF2 and NCK interacting kinase	TNIK	-0.65	0.64	<0.0001	<0.0001	-1.35	0.39	<0.0001	<0.0001	No	No
205730_s_at	actin binding LIM protein family, member 3	ABLIM3	-0.65	0.64	<0.0001	<0.0001	-1.21	0.43	<0.0001	<0.0001	No	No
213603_s_at	ras-related C3 botulinum toxin substrate 2 (rho family, small GTP binding protein Rac2)	RAC2	-0.64	0.64	<0.0001	<0.0001	-0.58	0.67	<0.0001	<0.0001	No	No
212364_at	myosin IB	MYO1B	-0.64	0.64	0.0006	<0.0001	-0.5	0.71	<0.0001	0.0011	No	No
206027_at	S100 calcium binding protein A3	S100A3	-0.64	0.64	0.0016	0.0017	-0.86	0.55	<0.0001	<0.0001	No	No
221043_at	---	---	-0.64	0.64	<0.0001	<0.0001	-0.41	0.75	0.0031	<0.0001	No	No
226481_at	Vpr (HIV-1) binding protein	VPRBP	-0.64	0.64	<0.0001	<0.0001	-0.64	0.64	<0.0001	<0.0001	No	No
219363_s_at	MTERF domain containing 1	MTERFD1	-0.64	0.64	<0.0001	<0.0001	-0.55	0.68	<0.0001	<0.0001	No	No
208634_s_at	microtubule-actin crosslinking factor 1	MACF1	-0.64	0.64	<0.0001	<0.0001	-0.74	0.6	<0.0001	<0.0001	No	No
226463_at	ATPase, H+ transporting, lysosomal 42kDa, V1 subunit C1	ATP6V1C1	-0.63	0.65	<0.0001	<0.0001	-0.34	0.79	0.0031	0.0011	No	No
203695_s_at	deafness, autosomal dominant 5	DFNA5	-0.63	0.65	<0.0001	<0.0001	-0.76	0.59	<0.0001	<0.0001	No	No
225846_at	epithelial splicing regulatory protein 1	ESRP1	-0.63	0.65	0.011	<0.0001	-1.86	0.28	<0.0001	<0.0001	No	No
218273_s_at	pyruvate dehyrogenase phosphatase catalytic subunit 1	PDP1	-0.63	0.65	<0.0001	<0.0001	-0.49	0.71	<0.0001	<0.0001	No	No
202294_at	stromal antigen 1	STAG1	-0.62	0.65	<0.0001	<0.0001	-0.64	0.64	<0.0001	<0.0001	No	No
223255_at	G2/M-phase specific E3 ubiquitin protein ligase	G2E3	-0.62	0.65	<0.0001	<0.0001	-0.55	0.68	<0.0001	<0.0001	No	No
210273_at	protocadherin 7	PCDH7	-0.62	0.65	0.0016	<0.0001	-0.51	0.7	<0.0001	0.0011	No	No
205527_s_at	gem (nuclear organelle) associated protein 4	GEMIN4	-0.62	0.65	<0.0001	<0.0001	-0.55	0.69	<0.0001	<0.0001	No	No
219188_s_at	MACRO domain containing 1	MACROD1	-0.61	0.65	0.0006	0.0005	-0.29	0.82	0.0069	0.0091	No	No
223599_at	tripartite motif-containing 6	TRIM6	-0.61	0.65	<0.0001	0.0017	-1.22	0.43	<0.0001	<0.0001	No	No
214771_x_at	myosin phosphatase Rho interacting protein	MPRIP	-0.61	0.66	0.0006	<0.0001	-0.68	0.63	<0.0001	<0.0001	No	No
212731_at	ankyrin repeat domain 46	ANKRD46	-0.6	0.66	<0.0001	<0.0001	-0.27	0.83	0.0006	0.0021	No	No
227307_at	tetraspanin 18	TSPAN18	-0.6	0.66	<0.0001	0.0005	-0.38	0.77	<0.0001	0.0011	Yes	No
229843_at	---	---	-0.6	0.66	0.0006	<0.0001	-0.54	0.69	<0.0001	<0.0001	No	No
226477_at	Vpr (HIV-1) binding protein	VPRBP	-0.6	0.66	0.0006	<0.0001	-0.62	0.65	<0.0001	<0.0001	No	No

204256_at	ELOVL family member 6, elongation of long chain fatty acids (FEN1/Elo2, SUR4/Elo3-like, yeast)	ELOVL6	-0.6	0.66	<0.0001	<0.0001	-0.63	0.65	<0.0001	<0.0001	No	No
233899_x_at	zinc finger and BTB domain containing 10	ZBTB10	-0.6	0.66	0.0016	0.0017	-0.72	0.61	<0.0001	<0.0001	No	No
222420_s_at	ubiquitin-conjugating enzyme E2H (UBC8 homolog, yeast)	UBE2H	-0.6	0.66	<0.0001	<0.0001	-0.59	0.67	<0.0001	<0.0001	Yes	No
225603_s_at	chromosome 8 open reading frame 83	C8orf83	-0.6	0.66	<0.0001	<0.0001	-0.5	0.71	<0.0001	<0.0001	No	No
218521_s_at	ubiquitin-conjugating enzyme E2W (putative)	UBE2W	-0.6	0.66	<0.0001	<0.0001	-0.36	0.78	<0.0001	0.0011	No	No
227856_at	chromosome 4 open reading frame 32	C4orf32	-0.59	0.66	<0.0001	<0.0001	-1.28	0.41	<0.0001	<0.0001	No	No
229103_at	wingless-type MMTV integration site family, member 3	WNT3	-0.59	0.66	<0.0001	<0.0001	-0.38	0.77	<0.0001	0.0021	No	No
229029_at	calcium/calmodulin-dependent protein kinase IV	CAMK4	-0.59	0.66	<0.0001	<0.0001	-0.42	0.75	0.0006	0.0021	No	No
209505_at	nuclear receptor subfamily 2, group F, member 1	NR2F1	-0.59	0.67	<0.0001	0.053	-0.76	0.59	<0.0001	<0.0001	No	No
217914_at	two pore segment channel 1	TPCN1	-0.59	0.67	<0.0001	<0.0001	-0.35	0.79	<0.0001	<0.0001	Yes	No
208697_s_at	eukaryotic translation initiation factor 3, subunit E	EIF3E	-0.58	0.67	<0.0001	<0.0001	-0.5	0.71	<0.0001	<0.0001	No	No
222496_s_at	RNA binding motif protein 47	RBM47	-0.58	0.67	0.0006	0.0005	-1.02	0.49	<0.0001	<0.0001	No	No
201533_at	catenin (cadherin-associated protein), beta 1, 88kDa	CTNNB1	-0.58	0.67	<0.0001	<0.0001	-0.32	0.8	0.0069	0.0224	No	No
203568_s_at	tripartite motif-containing 38	TRIM38	-0.58	0.67	<0.0001	<0.0001	-0.46	0.73	0.0031	0.0011	No	No
200918_s_at	signal recognition particle receptor (docking protein)	SRPR	-0.58	0.67	<0.0001	<0.0001	-0.61	0.66	<0.0001	<0.0001	No	No
235780_at	protein kinase, cAMP-dependent, catalytic, beta	PRKACB	-0.58	0.67	0.0006	<0.0001	-0.3	0.81	0.0372	0.058	No	No
215299_x_at	sulfotransferase family, cytosolic, 1A, phenol-preferring, member 1	SULT1A1	-0.57	0.67	<0.0001	<0.0001	-0.42	0.75	<0.0001	<0.0001	No	No
232546_at	tumor protein p73	TP73	-0.57	0.67	0.0009	0.0005	-0.39	0.76	0.0006	0.0011	No	No

208309_s_at	mucosa associated lymphoid tissue lymphoma translocation gene 1	MALT1	-0.57	0.67	<0.0001	0.0005	-0.77	0.59	<0.0001	<0.0001	No	No
227802_at	RUN and FYVE domain containing 3	RUFY3	-0.57	0.67	0.0049	0.0059	-0.56	0.68	<0.0001	<0.0001	No	No
208994_s_at	peptidylprolyl isomerase G (cyclophilin G)	PPIG	-0.57	0.67	0.0006	<0.0001	-0.69	0.62	<0.0001	<0.0001	No	No
202905_x_at	nibrin	NBN	-0.57	0.67	<0.0001	<0.0001	-0.51	0.7	<0.0001	0.0011	No	No
221636_s_at	MOCO sulphurase C-terminal domain containing 2	MOSC2	-0.56	0.68	<0.0001	<0.0001	-0.59	0.67	<0.0001	<0.0001	No	No
213044_at	Rho-associated, coiled-coil containing protein kinase 1	ROCK1	-0.56	0.68	<0.0001	<0.0001	-0.56	0.68	<0.0001	<0.0001	No	No
219038_at	MORC family CW-type zinc finger 4	MORC4	-0.56	0.68	<0.0001	<0.0001	-0.59	0.67	<0.0001	<0.0001	No	No
221020_s_at	solute carrier family 25, member 32	SLC25A32	-0.56	0.68	<0.0001	<0.0001	-0.48	0.72	<0.0001	<0.0001	No	No
210018_x_at	mucosa associated lymphoid tissue lymphoma translocation gene 1	MALT1	-0.56	0.68	<0.0001	0.0005	-0.71	0.61	<0.0001	<0.0001	No	No
229014_at	hypothetical LOC441094	FLJ42709	-0.56	0.68	0.0006	<0.0001	-0.88	0.55	<0.0001	<0.0001	No	No
215222_x_at	microtubule-actin crosslinking factor 1	MACF1	-0.56	0.68	0.0006	<0.0001	-0.79	0.58	<0.0001	<0.0001	No	No
235692_at	SH3-domain kinase binding protein 1	SH3KBP1	-0.56	0.68	<0.0001	0.0005	-0.55	0.68	<0.0001	<0.0001	No	No
218035_s_at	RNA binding motif protein 47	RBM47	-0.55	0.68	<0.0001	<0.0001	-0.89	0.54	<0.0001	<0.0001	No	No
239814_at	hypothetical LOC100506860	LOC100506 860	-0.55	0.68	0.0049	<0.0001	-0.61	0.66	<0.0001	<0.0001	No	No
231697_s_at	---	---	-0.55	0.68	0.0049	<0.0001	-0.67	0.63	<0.0001	<0.0001	No	No
203584_at	tetratricopeptide repeat domain 35	TTC35	-0.55	0.68	0.0009	0.0005	-0.45	0.73	<0.0001	<0.0001	No	No
230047_at	Rho GTPase activating protein 42	ARHGAP42	-0.55	0.68	0.0009	<0.0001	-0.89	0.54	<0.0001	<0.0001	No	No
235696_at	---	---	-0.55	0.68	<0.0001	<0.0001	-0.52	0.7	<0.0001	<0.0001	No	No
208763_s_at	TSC22 domain family, member 3	TSC22D3	-0.55	0.68	<0.0001	<0.0001	-0.22	0.86	0.1715	0.058	No	No
243000_at	cyclin-dependent kinase 6	CDK6	-0.55	0.69	<0.0001	<0.0001	-0.41	0.76	<0.0001	<0.0001	No	No
224937_at	prostaglandin F2 receptor negative regulator	PTGFRN	-0.55	0.69	<0.0001	<0.0001	-0.23	0.85	0.0069	0.0224	No	No
223258_s_at	G2/M-phase specific E3 ubiquitin protein ligase	G2E3	-0.54	0.69	<0.0001	<0.0001	-0.57	0.68	<0.0001	<0.0001	No	No

208993_s_at	peptidylprolyl isomerase G (cyclophilin G)	PPIG	-0.54	0.69	<0.0001	<0.0001	-0.67	0.63	<0.0001	<0.0001	No	No
205308_at	family with sequence similarity 164, member A	FAM164A	-0.54	0.69	0.0006	<0.0001	-0.56	0.68	<0.0001	<0.0001	No	No
212197_x_at	myosin phosphatase Rho interacting protein	MPRIP	-0.54	0.69	0.0006	<0.0001	-0.82	0.57	<0.0001	<0.0001	No	No
213229_at	dicer 1, ribonuclease type III	DICER1	-0.54	0.69	0.0016	0.0005	-0.51	0.7	<0.0001	<0.0001	No	No
228489_at	transmembrane 4 L six family member 18	TM4SF18	-0.53	0.69	0.0006	<0.0001	-0.91	0.53	<0.0001	<0.0001	Yes	No
204274_at	estrogen receptor binding site associated, antigen, 9	EBAG9	-0.53	0.69	<0.0001	<0.0001	-0.59	0.67	<0.0001	<0.0001	No	No
203411_s_at	lamin A/C	LMNA	-0.53	0.69	<0.0001	<0.0001	-0.47	0.72	<0.0001	<0.0001	No	No
209607_x_at	sulfotransferase family, cytosolic, 1A, phenol-preferring, member 3 /// sulfotransferase family, cytosolic, 1A, phenol-preferring, member 4	SULT1A3 /// SULT1A4	-0.53	0.69	<0.0001	<0.0001	-0.38	0.77	0.0006	0.0021	No	No
226715_at	forkhead box K1	FOXK1	-0.53	0.69	0.0006	<0.0001	-0.37	0.78	0.0006	0.0039	No	No
210878_s_at	lysine (K)-specific demethylase 3B	KDM3B	-0.53	0.69	<0.0001	<0.0001	-0.42	0.75	<0.0001	<0.0001	No	No
230944_at	chromosome 6 open reading frame 223	C6orf223	-0.53	0.69	0.0049	<0.0001	-0.53	0.69	<0.0001	<0.0001	Yes	No
210117_at	sperm associated antigen 1	SPAG1	-0.53	0.69	<0.0001	<0.0001	-0.61	0.66	<0.0001	0.0011	No	No
222572_at	pyruvate dehyrogenase phosphatase catalytic subunit 1	PDP1	-0.53	0.69	0.0006	<0.0001	-0.38	0.77	<0.0001	<0.0001	No	No
224729_s_at	ATP synthase mitochondrial F1 complex assembly factor 1	ATPAF1	-0.52	0.7	<0.0001	<0.0001	-0.37	0.78	0.0006	0.0021	No	No
218549_s_at	family with sequence similarity 82, member B	FAM82B	-0.52	0.7	0.0006	<0.0001	-0.72	0.61	<0.0001	<0.0001	No	No
225299_at	myosin VB	MYO5B	-0.52	0.7	<0.0001	<0.0001	-0.38	0.77	0.0006	0.0021	No	No
201772_at	antizyme inhibitor 1	AZIN1	-0.52	0.7	<0.0001	<0.0001	-0.45	0.73	0.0006	0.0011	No	No
228065_at	B-cell CLL/lymphoma 9-like	BCL9L	-0.52	0.7	0.0006	<0.0001	-0.29	0.82	0.0069	0.0021	Yes	No
1558111_at	muscleblind-like (Drosophila)	MBNL1	-0.52	0.7	<0.0001	<0.0001	-0.93	0.52	<0.0001	<0.0001	No	No
231270_at	carbonic anhydrase XIII	CA13	-0.52	0.7	0.0006	<0.0001	-0.44	0.74	<0.0001	<0.0001	No	No
204278_s_at	estrogen receptor binding site associated, antigen, 9	EBAG9	-0.52	0.7	<0.0001	<0.0001	-0.6	0.66	<0.0001	<0.0001	No	No

204321_at	neogenin 1	NEO1	0.68	1.6	0.011	<0.0001	0.64	1.56	<0.0001	<0.0001	No	No
211684_s_at	dynein, cytoplasmic 1, intermediate chain 2	DYNC1I2	0.68	1.61	<0.0001	<0.0001	0.59	1.51	<0.0001	<0.0001	No	No
1554418_s_at	sparc/osteonectin, cwcv and kazal-like domains proteoglycan (testican) 3	SPOCK3	0.69	1.62	0.0016	0.0017	0.47	1.39	0.0006	<0.0001	No	No
232202_at	---	---	0.69	1.62	<0.0001	<0.0001	0.21	1.15	0.0372	0.058	No	No
221973_at	hypothetical LOC100506076 /// hypothetical LOC100506123	LOC100506076 /// LOC100506123	0.7	1.62	<0.0001	<0.0001	0.67	1.59	<0.0001	<0.0001	No	No
224657_at	ERBB receptor feedback inhibitor 1	ERRFI1	0.7	1.63	<0.0001	<0.0001	0.58	1.5	<0.0001	<0.0001	No	No
1557302_at	zinc finger protein 585B	ZNF585B	0.7	1.63	<0.0001	<0.0001	1.06	2.08	<0.0001	<0.0001	No	No
226186_at	tropomodulin 2 (neuronal)	TMOD2	0.7	1.63	0.0049	<0.0001	0.62	1.53	0.0031	0.0021	No	No
214036_at	ephrin-A5	EFNA5	0.71	1.64	0.0004	<0.0001	0.48	1.4	<0.0001	<0.0001	Yes	No
225706_at	glucocorticoid induced transcript 1	GLCCI1	0.72	1.65	<0.0001	<0.0001	0.56	1.47	<0.0001	<0.0001	No	No
204014_at	dual specificity phosphatase 4	DUSP4	0.73	1.66	<0.0001	<0.0001	0.86	1.81	<0.0001	<0.0001	No	No
229555_at	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 5 (GalNAc-T5)	GALNT5	0.75	1.68	<0.0001	<0.0001	0.49	1.4	<0.0001	<0.0001	No	No
222127_s_at	exocyst complex component 1	EXOC1	0.76	1.69	<0.0001	<0.0001	0.75	1.68	<0.0001	<0.0001	No	No
209197_at	synaptotagmin XI	SYT11	0.77	1.71	<0.0001	<0.0001	0.86	1.81	<0.0001	<0.0001	No	No
223251_s_at	ankyrin repeat domain 10	ANKRD10	0.82	1.76	<0.0001	<0.0001	1.01	2.01	<0.0001	<0.0001	No	No
228188_at	FOS-like antigen 2	FOSL2	0.83	1.77	<0.0001	<0.0001	0.67	1.59	<0.0001	<0.0001	No	No
200731_s_at	protein tyrosine phosphatase type IVA, member 1	PTP4A1	0.84	1.79	<0.0001	<0.0001	0.58	1.49	<0.0001	<0.0001	No	No
202388_at	regulator of G-protein signaling 2, 24kDa	RGS2	0.86	1.82	<0.0001	<0.0001	0.52	1.44	<0.0001	<0.0001	No	No
217764_s_at	RAB31, member RAS oncogene family	RAB31	0.87	1.83	<0.0001	<0.0001	0.29	1.22	0.0069	0.0011	No	No
1559360_at	---	---	0.88	1.84	0.0004	<0.0001	0.68	1.6	<0.0001	<0.0001	No	No
205352_at	serpin peptidase inhibitor, clade I (neuroserpin), member 1	SERPINI1	0.88	1.84	<0.0001	<0.0001	0.66	1.58	<0.0001	<0.0001	No	No
206059_at	zinc finger protein 91	ZNF91	0.88	1.85	<0.0001	<0.0001	0.95	1.93	<0.0001	<0.0001	No	No
212412_at	PDZ and LIM domain 5	PDLIM5	0.88	1.85	<0.0001	<0.0001	0.96	1.94	<0.0001	<0.0001	No	No
226939_at	cytoplasmic polyadenylation element binding protein 2	CPEB2	0.88	1.85	<0.0001	<0.0001	0.58	1.49	<0.0001	<0.0001	No	No

204015_s_at	dual specificity phosphatase 4	DUSP4	0.89	1.86	<0.0001	<0.0001	0.72	1.65	<0.0001	<0.0001	No	No
227230_s_at	KIAA1211	KIAA1211	0.9	1.86	<0.0001	<0.0001	0.47	1.39	<0.0001	<0.0001	No	No
211745_x_at	hemoglobin, alpha 1 /// hemoglobin, alpha 2	HBA1 /// HBA2	0.9	1.87	<0.0001	<0.0001	0.64	1.56	<0.0001	<0.0001	No	No
206271_at	toll-like receptor 3	TLR3	0.91	1.88	<0.0001	<0.0001	0.29	1.22	0.0069	0.0021	No	No
218248_at	family with sequence similarity 111, member A	FAM111A	0.91	1.88	0.011	<0.0001	1.4	2.63	<0.0001	<0.0001	No	No
233537_at	keratin associated protein 3-1	KRTAP3-1	0.92	1.89	0.0009	<0.0001	1.97	3.91	<0.0001	<0.0001	No	No
217414_x_at	hemoglobin, alpha 1 /// hemoglobin, alpha 2	HBA1 /// HBA2	0.94	1.91	<0.0001	<0.0001	0.52	1.43	0.0006	0.0011	No	No
236129_at	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 5 (GalNAc-T5)	GALNT5	0.97	1.95	<0.0001	<0.0001	0.34	1.27	0.0006	0.0011	No	No
203243_s_at	PDZ and LIM domain 5	PDLIM5	0.97	1.96	<0.0001	<0.0001	1.19	2.29	<0.0001	<0.0001	No	No
212665_at	TCDD-inducible poly(ADP-ribose) polymerase	TIPARP	1.02	2.03	<0.0001	<0.0001	0.81	1.75	<0.0001	<0.0001	No	No
200897_s_at	palladin, cytoskeletal associated protein	PALLD	1.06	2.09	<0.0001	<0.0001	1	1.99	<0.0001	<0.0001	Yes	No
211681_s_at	PDZ and LIM domain 5	PDLIM5	1.09	2.14	<0.0001	<0.0001	1.15	2.22	<0.0001	<0.0001	No	No
221994_at	PDZ and LIM domain 5	PDLIM5	1.14	2.21	<0.0001	<0.0001	1.24	2.37	<0.0001	<0.0001	No	No
204790_at	SMAD family member 7	SMAD7	1.22	2.34	<0.0001	<0.0001	0.91	1.88	<0.0001	<0.0001	No	No
215596_s_at	listerin E3 ubiquitin protein ligase 1	LTN1	1.23	2.35	<0.0001	<0.0001	2.24	4.74	<0.0001	<0.0001	No	No
203242_s_at	PDZ and LIM domain 5	PDLIM5	1.25	2.38	<0.0001	<0.0001	1.02	2.03	<0.0001	<0.0001	No	No
217763_s_at	RAB31, member RAS oncogene family	RAB31	1.26	2.4	<0.0001	<0.0001	0.58	1.5	<0.0001	<0.0001	No	No
200907_s_at	palladin, cytoskeletal associated protein	PALLD	1.3	2.45	<0.0001	<0.0001	1.09	2.13	<0.0001	<0.0001	Yes	No
216804_s_at	PDZ and LIM domain 5	PDLIM5	1.33	2.52	<0.0001	<0.0001	1.1	2.15	<0.0001	<0.0001	No	No
214414_x_at	hemoglobin, alpha 1 /// hemoglobin, alpha 2	HBA1 /// HBA2	1.34	2.53	<0.0001	<0.0001	1.07	2.1	<0.0001	<0.0001	No	No
200906_s_at	palladin, cytoskeletal associated protein	PALLD	1.37	2.59	<0.0001	<0.0001	1.01	2.01	<0.0001	<0.0001	Yes	No
213684_s_at	PDZ and LIM domain 5	PDLIM5	1.56	2.95	<0.0001	<0.0001	1.38	2.6	<0.0001	<0.0001	No	No
215767_at	zinc finger protein 804A	ZNF804A	1.61	3.05	<0.0001	<0.0001	1.86	3.62	<0.0001	<0.0001	No	No
203708_at	phosphodiesterase 4B, cAMP-specific	PDE4B	1.66	3.16	<0.0001	<0.0001	-2	0.25	<0.0001	<0.0001	No	No
200633_at	ubiquitin B	UBB	2.11	4.32	<0.0001	<0.0001	1.28	2.43	<0.0001	<0.0001	No	No

204035_at	secretogranin II	SCG2	2.16	4.46	<0.0001	<0.0001	2.38	5.2	<0.0001	<0.0001	No	No
206172_at	interleukin 13 receptor, alpha 2	IL13RA2	2.9	7.46	<0.0001	<0.0001	0.71	1.64	<0.0001	<0.0001	No	No

[a]Results from triplicates for each sample (two separate experiments: parental versus MLL2-/- #1 and #2; parental versus MLL2-/- #3 and #4). [b]False discovery rate was calculated using Significance Analysis of Microarrays (SAM). [c]Identified by ChIP (citation). [d]Probes that displayed a down-regulation of 50% or more in the first experiment and were confirmed to be significantly down-regulated in the second experiment were used for IPA.

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